Medxplore: Frontiers in Medical Science

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This book is a part of the "**Periodic Series in Multidisciplinary Studies**", designed to showcase interdisciplinary research and academic contributions from various fields including science, humanities, technology, education, and more.

The goal of this series is to create a platform for both established and emerging scholars to present their findings in a way that transcends traditional academic silos. By promoting interdisciplinary collaboration and integrated thinking, the series contributes to the advancement of knowledge and the resolution of complex global challenges that require multi-perspective approaches. We believe that sharing diverse voices and research methodologies can catalyse meaningful progress across fields and foster a more informed and connected scholarly community.

This volume offers unique insights and case studies contributed by experts and researchers from around the world. Each chapter reflects the authors' individual perspectives and scholarly expertise. Readers are encouraged to engage critically with the content, reflect on the findings, and explore how these insights may apply to their own fields of interest or professional practice.

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Preface of the Series

In an era where medical science is experiencing unprecedented growth, this book series endeavors to capture the dynamic interplay between foundational research and its clinical applications. The rapid advancements in genomics, immunotherapy, and precision medicine are reshaping our understanding of human health and disease. As we stand on the cusp of transformative breakthroughs, this series aims to provide a comprehensive overview of the current landscape, challenges, and future directions in medical science.

The integration of disciplines such as biomedicine, medical physics, and translational research has paved the way for innovations that were once the realm of science fiction. From the application of CRISPR technology in gene editing to the development of mRNA vaccines, the boundaries of what is possible are continually expanding. This series seeks to illuminate these developments, offering insights into how basic scientific discoveries are being translated into tangible therapies and interventions.

As we delve into the complexities of medical science, it is essential to recognize the ethical considerations and societal implications that accompany these advancements. The pursuit of knowledge must be balanced with a commitment to equity, accessibility, and the well-being of all individuals. This series encourages readers to engage critically with the content, fostering a deeper understanding of the responsibilities that come with scientific progress.

Through this compilation, we invite readers to explore the multifaceted world of medical science, appreciating both its achievements and the ongoing challenges that drive its evolution. It is our hope that this series will serve as a valuable resource for students, professionals, and anyone interested in the ever-evolving field of medical science.

Editor of the Series

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Chapter-I

TARGETING TUMOR MICROENVIRONMENT IN METASTATIC CANCER

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Abstract--- Cancer is the second leading cause of global mortality, accounting for approximately one in six deaths worldwide, with an estimated 9.6 million deaths predicted in 2018. Disproportionately, 70% of cancer-related deaths occur in low- and middle-income countries. At its core, cancer is characterized by the uncontrolled, abnormal growth of cells, resulting from accumulated DNA alterations, which ultimately form a tumor. Accumulated oncogenic mutations allow tumor cells to proliferate and migrate without restriction, aid in invasion and colonization, trigger widespread angiogenesis, and spread. Malignant neoplasms, another name for cancer, are characterized by unchecked cell proliferation that has the capacity to penetrate and spread to other organs. Breast cancer is the most frequent cancer in women and the second leading cause of death globally, accounting for 9.6 million deaths in 2018. The number of instances is expected to rise in the near future. Recent studies have shown that the overexpression of metastasis-associated protein (MTA1), which stimulates angiogenesis, is closely linked to the metastasis of breast cancer and other malignant tumors. The use of the tumor microenvironment as a therapeutic target for cancer has greatly increased in both research and clinical interest. Here, we highlight the difficulties in focusing on the tumor microenvironment to attain therapeutic efficacy and provide an overview of the recent developments in this area in both drug development and clinical trials. address ways to influence the pro-tumor milieu and optimize therapeutic outcomes, as well as investigate novel technologies and methodologies to better understand the tumor microenvironment.

Keywords--- Drug, Resistance, Target, Therapy, Tumor Microenvironment.

1. INTRODUCTION

MTA1, a gene associated with tumor metastasis, is known to regulate other metastasis-related genes. Higher MTA1 levels are linked to several tumor types. Unfortunately, because TNBC patients lack ER, PR, and HER2/Neu receptors, their therapy options are limited, and they have the worst prognosis. Positively, chemotherapy usually works well for this kind of breast cancer. Additionally, angiogenesis is necessary for the promotion and maintenance of tumor growth in malignant tumors that are growing aggressively (Anderson & Simon, 2020). Consequently, it is well known that anti-angiogenic therapy in conjunction with chemotherapy is a crucial cancer treatment approach. Consequently, there is a great demand for novel anti-cancer drugs with few adverse effects.

In this study, we examined the impact of several new chemicals, both synthetic and derived from plants, on their ability to prevent cancer. The Soxhlet extraction method was used to purify Dioscorea bulbifera root extracts in an activity-guided manner. Only the ethyl acetate, acetone, and methanol extract out of the eight solvent extracts were determined to be the most promising. Additionally, we created nine new benzisoxazole (7a-i) compounds and evaluated their potential for biological activity. 3- (1- ((3- (3- (Benzyloxy) -4-methoxyphenyl) -4, 5- dihydroisoxazole- 5- yl) methyl) piperidine-4-yl)6-fluorobenzo[d] isoxazole (7e) was determined to be the most promising of the synthesized compounds. The chemical (7e) and D. bulbifera active extracts significantly inhibited the migration and proliferation of breast cancer cells stimulated by recombinant MTA1. It is well established that MTA1-protein is essential for both angiogenesis and cancer. In order to serve as a positive inducer in each experiment, a recombinant MTA1 protein was produced and purified. In vivo, the chosen drugs demonstrated angio-inhibitory and anti-tumor effects. Additionally, nuclear labeling and flow cytometry analysis were used to visualize the inducing apoptotic process. These results demonstrate that D. bulbifera root active extracts have the potential to be a rich source of bioactive compounds, and compound 7e's reported biological activity makes it an appealing therapeutic candidate.

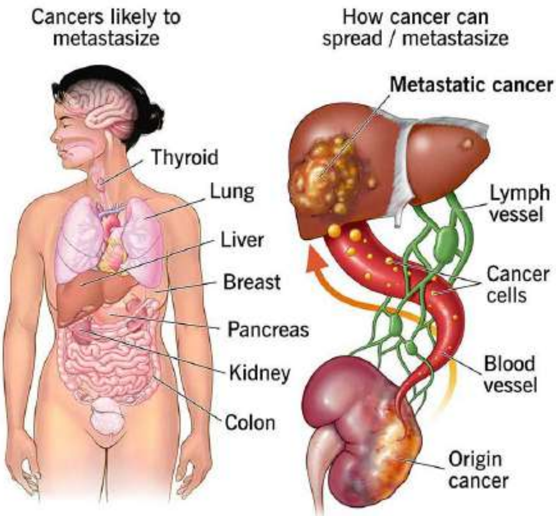

Figure 1: Metastatic Cancer (source: web)

When combined, our findings show that MTA1 promotes breast cancer survival and progression. For this reason, the third objective involved using RNA interference technology to silence the MTA1 gene and creating stable transfected clones with MTA1-shRNA using the mammalian transfection vector pCDNA-3. Using western blotting, immunofluorescence, and qPCR analysis, the complete suppression of MTA1 expression in silenced clones was confirmed. Additionally, the migratory ability of these clones was evaluated using the scratch assay, where MTA1-shRNA successfully blocked the wound closing while the scramble wound was fully closed. Additionally, it was clear from real-time PCR study of EMT regulatory genes that MTA1 knockdown resulted in lower expressions of EMT transcription factors TWIST and SNAIL and increased expression of E-cadherin. These results support novel strategies for the treatment of breast cancer (Aswathy et al., 2020).

2. CANCER AND ITS BRIEF HISTORY

Hallmarks of Cancer

According to Hanahan and Weinberg (2000), cancer cells are generally distinguished by their capacity to produce their own growth signals, disregard growth-inhibitory signals, prevent cell death, multiply endlessly, maintain angiogenesis, and infiltrate tissues through capillary walls and basement membranes. Finally, one of the characteristics of cancer is the avoidance of immunosurveillance (Dunn, Old, & Schreiber, 2004). When a tumor does not spread to the surrounding tissues, it is considered benign. On the other hand, the tumor is considered malignant if it spreads to distant tissues and invades nearby tissues (Almeida & Barry, 2011). The etiology of certain tumors is not fully understood (Kilmister et al., 2022). Nonetheless, a number of risk factors that increase an individual's vulnerability to cancer have been found. According to the National Cancer Institute, these include heavy smoking, exposure to UV rays, drinking alcohol, eating poorly, being sedentary and obese, having chronic infections, having a family history and genetic predisposition, having hormones, and being around dangerous substances or radiation. A limitless supply of chemically varied chemicals can be obtained from natural plant products, either as pure compounds or as plant extracts.

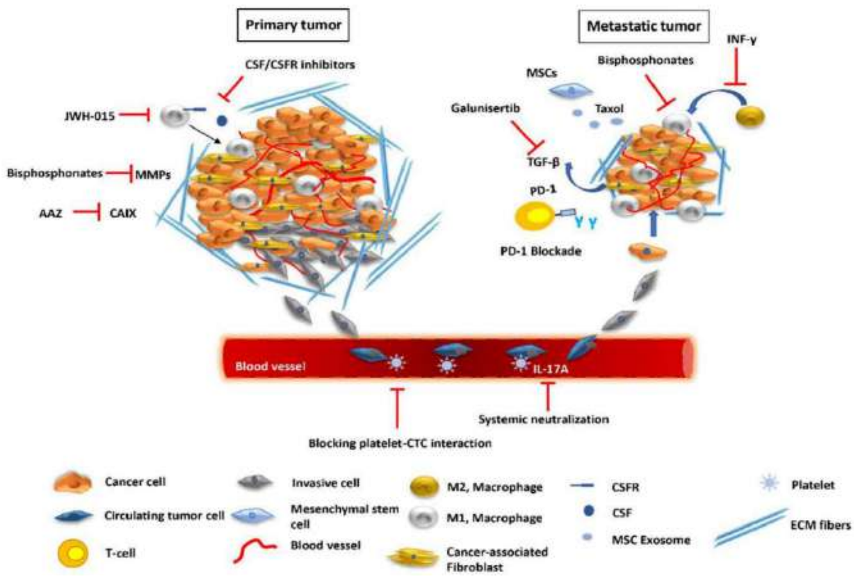

Figure 2: Metastatic Tumour Environment (source: web)

Traditional medicine continues to be the mainstay of medical care in Asia and other regions of the world (Greenwell & Rahman, 2015a). Numerous compounds found in plants are used to cure a variety of illnesses. As a result, studies have been conducted to look into the possible anti-cancer properties of many plants that are utilized in traditional herbal therapy. Plant phytochemicals have a solid track record of being used to treat cancer. A study claims that over half of the anticancer medications on the market come from plant origins (Shaikh, Pund, Dawane, & Iliyas, 2014). However, because chemotherapy resistance is a significant barrier to effective treatment, they are linked to negative side effects, and often, the effects are short-lived. Therefore, to lessen the health burden of this debilitating condition, there is a great need for novel anticancer drugs with few adverse effects. The air yam, or Dioscorea bulbifera, is a traditional medicinal plant that is classified within the Dioscoraeceae family. It contains the steroidal saponin diosgenin and is composed of organic acids and polyphenols, some of which may be antioxidants.

Transformation and Isolation of Plasmid pGEX-5X-2-MTA1

The MTA1 insert-containing plasmid pGEX-5X-2 was obtained by aseptically inoculating fresh cultures or transforming competent cells from glycerol stocks. The transformation of chemically competent E. coli followed the manufacturer's instructions. One vial of cells was briefly thawed on ice. The bottle was then gently combined with 100ng of the plasmid DNA (pGEX-5X-2-MTA1). After 30 minutes of ice incubation, the cells received a brief heat shock treatment for 30 seconds at 42°C. The vial was immediately put on ice for two minutes. A Qiagen kit from Germany was used to further inoculate a subset of colonies from each plate in order to isolate the plasmid. In short, 500ml of an overnight LB culture was centrifuged at 4000g for 10 minutes in order to extract cells. After that, the pellet was reconstituted in Buffer (P2) with RNase A already added. After that, the cell suspension was lysed for five minutes at room temperature using Lysis Buffer (P3). After centrifuging the mixture for 10 minutes at 12000g, the clear supernatant was transferred to a column that had been pre-equilibrated with QBT buffer and drained using gravity flow. Wash Buffer (QC) was used to clean the column after the lysate filtration process was finished. Elution Buffer (QF) was then added to the column to elute the plasmid DNA (Belli et al., 2018). The DNA pellet was centrifuged at 15000g for 30 minutes at 4°C, and isopropanol was eliminated by washing it with 70% ethanol. After carefully removing the supernatant, the DNA pellet was allowed to air dry before being dissolved in the smallest amount of TE buffer. UV spectroscopy was used to evaluate the plasmid DNA's purity and concentration.

Agarose Gel Electrophoresis to Confirm Plasmid

TAE buffer (39 mM Tris, Acetic Acid, 0.5 M EDTA) was used to make a 0.8% Agarose solution. Ethidium bromide (0.5μg/ml) was then added to the solution and left to settle. The gel was put in the TAE buffer-filled electrophoresis tank. In a different lane, 1μg of plasmid and a conventional 1 kb DNA ladder were added. The gel was recorded and the electrophoresis was run at 50V.

Expression and Purification of Recombinant MTA1-Protein

IPTG induction was used to produce MTA1 protein in BL21 strains of E. Coli culture that carried the plasmid pGEX-5x-2-MTA1. After being inoculated into 5 milliliters of LB media, transformed colonies were cultured with ampicillin-containing media for the entire night. After moving the culture into bulk media and growing it till the OD reached 0.3, 50μM IPTG was added, and the shaker incubator was left to incubate it overnight. Processing of the expressed protein downstream: The culture was centrifuged for 10 minutes at 40C at 6000 rpm. PBS with a protease inhibitor cocktail and triton X-100 was used to resuspend the pellet. After being sonicated, the liquid was centrifuged. After centrifuging once again, the obtained supernatant was combined with PBS that included 2% triton X-100 N-Lauryl sarcosine and protease inhibitor. Protein-containing supernatant was measured and put through an affinity column for further purification.

Purification of MTA1-Protein by GST Tag Column

Glutathione S-transferase (GST) tag, which possesses affinity for recombinant MTA1-protein, is present in the affinity column. The binding buffer was used to equilibrate the column. Using 1% triton X-100, the bacterially expressed crude protein was put onto the column. Binding buffer was used to re-equilibrate the column until the OD reached 0.05. All of the unattached proteins are eliminated in this stage. Next, elution buffer containing reduced glutathione was used to elute the bound protein sample, and fractions were collected until the optical density (OD) reached zero. The absorbance at 280 nm was used to pool all of the peak fractions.

SDS-PAGE and Detection by Silver Staining

To perform SDS-PAGE, a 1 mm thick polyacrylamide gel was prepared. A gel cassette was made up of three vacuum-greased spacers that separated two glass plates that were 1 mm thick. They clamped the glass panels together. A resolving gel was put into the gel cassette, and then a stacking gel was put on top. Ten microliters of TEMED were added to the mixture. A plastic comb was placed into the stacking gel to create slots for loading the samples after the contents had been poured over the resolving gel. Once the gel had polymerized, the comb was taken out. After removing the bottom spacer, the glass cassette was put onto the electrophoretic device. The running buffer was added to the electrophoresis chamber. Protein samples were heated to a boiling water bath for 5 to 8 minutes after being combined with 5X sample buffer with mercaptothions and bromophenol blue.

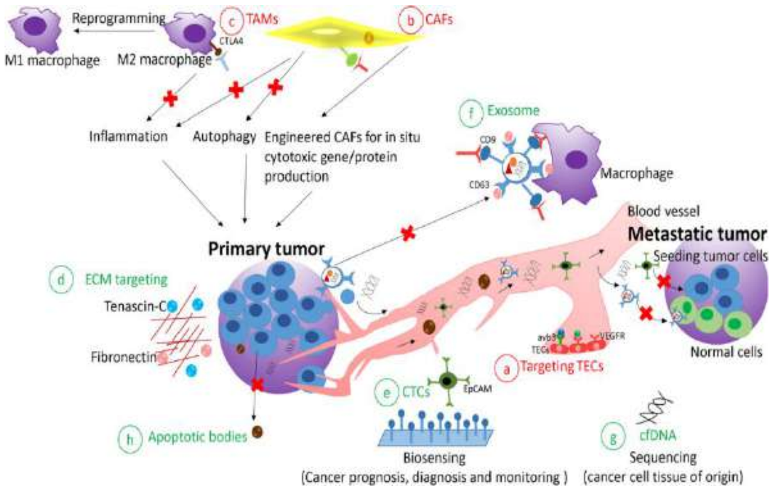

Figure 3: Tumor Targeting Environment (source: web)

Silver Staining

The gel was fixed overnight in a fixative solution (50µl formaldehyde, 12% acetic acid, and 50% v/v ethanol) following electrophoresis. After being pre-treated with 0.02% sodium thiosulphate for two minutes, they were then rinsed three times in distilled water after being cleaned three times with 50% ethanol for 20 minutes each. After 45 minutes of soaking in a 0.2% silver nitrate solution with 75µl of formaldehyde solution, the gel was carefully cleaned with distilled water. After developing the gel in a developing buffer (6% sodium carbonate and 50µl formaldehyde), the reaction was stopped with acetic acid after protein bands were visible.

Western Blotting

In short, Lowry's protein estimate was used to quantify the concentration of the recombinant protein MTA1 following purification. A protein sample of about 50 µg was separated on a 12.5% SDS-PAGE, electrotransferred to a PVDF membrane, and then left overnight in blocking buffer (5% skim milk solution). The following day, it was incubated for two hours with a 1:500 dilution of the anti-MTA1 primary antibody, and then with a 1:5000 dilution of the HRP-tagged secondary antibody. The Enhanced Chemi Luminescence (ECL) method (Bio-Rad, USA) was used to create the immunoblot in a BioRad Gel document (Tsai et al., 2014).

Trypan Blue Dye is Used in an Exclusion Experiment for Different Solvent Extracts

24 well culture plates with full DMEM medium were planted with 1 x104 cells per well. After 12 hours, the media was swapped out with a full medium that contained various chemicals (plant extracts 100µg/ml and benzoisoxazole derivatives (7a-i) 100µM) and the corresponding controls. The incubator was then humidified and kept at 370C with 5% CO2. Following incubation, cells were extracted using centrifugation for five minutes at 3000 RPM.

3. MTT ASSAY

After being seeded with full DMEM media in a 96-well tissue culture plate (Nunc MicroWellTM), MDA-MB-231 cells (3x10^4) after 4 hours of incubation at 37°C. Medium blanks, treatment controls, and vehicle controls (0.1% DMSO) were all used in triplicate for the assay.

Proliferation Assay

After that, 10 ng of MTA1 protein and various solvent extracts of D. bulbifera (100 g/ml) and compound 7e (1, 5, 10, 50, and 100 mM) were given to the cells individually or in combination. The mixture was left to incubate for 48 hours following the addition of 3 [H]-thymidine (1 Ci/ml). Trypsinization of the cells occurred following a wash with PBS. 10% (w/v) trichloroacetic acid and one milliliter of cold water were used to treat the cell pellet. Following the extraction of DNA Radioactivity was measured in each sample after five millilitres of scintillation cocktail fluid were added. The percentage of triplicate data presented as the control mean SEM.

Wound Healing Assay

After being seeded in six-well plates, the cells were cultivated to confluence. Mitomycin-C (10 ng/ml) was given to the cells for three hours before the chemicals were added. A new tip was used to make a small scratch when the cells were 80–90 percent confluent. Medium containing various solvent extracts of D. bulbifera (100 ng/ml) and compound 7e either alone or in combination with MTA1 protein (10 ng/ml) was added to the wells after the cells had been washed with PBS. After that, the cells underwent a full day of incubation. At a 20X magnification, photographs were taken with a Zeiss phase-contrast inverted microscope following incubation.

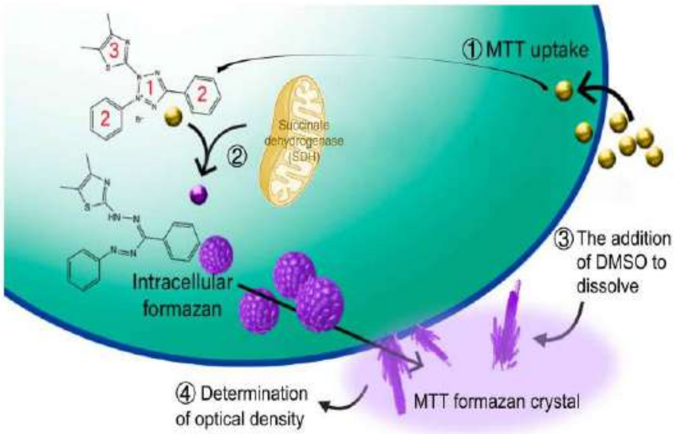

Figure 4: Cell Proliferation Inhibition Assay (source: web)

Peritoneal Angiogenesis in Vivo Assay

The test was conducted using an Ehrlich ascites tumor (EAT)-bearing mouse model to evaluate the anti-angiogenic effects of various solvent extracts and chemical 7e. Female Swiss albino mice were transplanted with EAT cells and treated with compound 7e and different solvent extracts daily from the sixth day onwards, with daily growth measurements recorded. The mice received intraperitoneal injections of EAT cells, and on the sixteenth day, the peritoneal cavity was examined for neovasculature, with photographs taken of the inner lining of the peritoneum to assess angiogenesis (Ji et al., 2013).

Cellular Staining for Apoptosis

It is likely that substances with the ability to alter planned cell death will be beneficial to medicine. In order to examine how D. bulbifera solvent extracts altered the morphology of EAT cells following treatment, Giemsa staining was employed. To sum up, cells from the in vivo test were extracted and washed with PBS after being treated and controlled with different solvent extracts (25 mg/kg b.w.). After being fixed in methanol and stained with a 10% Giemsa solution, the cells were examined at 40X magnification using an optical light microscope and recorded using a Zeiss AxioVert camera.

Flow Cytometric Analysis

In order to investigate the distribution of cell cycle phases, EAT cells (1x106 cells/ml) were subjected to treatment with or without the chemical 7e (100 M) for a range of times, including 0 hours, 45 minutes, 3 hours, and 6 hours. After the cells were taken, the cell cycle was looked at. For the overnight fixation at 40C, cells were quickly resuspended in ice-cold 70% ethanol. Following centrifugation, the cells were dyed with 10 g/ml propidium iodide, allowed to sit at room temperature for half an hour, and then rehydrated with PBS. The Beckman Coulter Cell Lab QuantaTM SC Flow Cytometer was utilized for the analysis.

4. APOPTOSIS USING NUCLEAR STAINING

Morphological changes in EAT cells undergoing apoptosis after treatment with chemical 7e were analyzed using dual labeling with acridine orange (AO) and ethidium bromide (EtBr). AO stains both living and dead cells, while EtBr specifically stains nuclei of cells with compromised membrane integrity and examined under a Zeiss AxioVert fluorescent microscope after staining with AO/EtBr, allowing for the imaging of apoptotic cells and detection of nuclei.

Chemotherapy is the most common treatment for cancer worldwide. Depending on the stage of the tumor, it may be combined with surgery or radiotherapy. Since the identification of a number of significant mutations that contribute to carcinogenesis. Even though it has become increasingly clear that the TME's influence is important for tumor growth and MDR, cancer research has focused on tumor cells. For a late-stage solid tumor, the tumor microenvironment is extremely diverse and intricate. First, the genomic profiles of the tumor cells alter the majority of the surrounding tissue. The rapid growth of the tumor cells initiates a number of processes, including TME adaptation to the altered environment and hypoxia-induced metabolic reprogramming. The inflammation that occurs at the tumor site frequently leads to the interaction of cancer cells with nearby cells like immune system cells and stromal cells. The extracellular matrix (ECM) is a complex, three-dimensional framework comprising collagen, elastin, fibronectin, hyaluronic acid, proteoglycans, and glycoproteins that provides structural support to tissues, maintains moisture and pH balance, and serves as a reservoir for growth factors. The heparanase inhibitor ronespartat (SST0001), which has a completed Phase 1 clinical trial for the treatment of multiple myeloma (Clinical Trial NCT01764880), had encouraging results in preventing tumor growth when administered either alone or in conjunction with other TME targeted medicines (Dmello et al., 2021). However, medicines that break down and/or deconstruct extracellular matrix (ECM) must be administered with caution due to the possibility that they will cause metastasis rather than halt the progression of the tumor.

5. THERAPEUTIC STRATEGIES TARGETING TME

For a long time, research on cancer has focused primarily on the cancerous tumor cells themselves, with little attention paid to the significance of the tumor microenvironment (TME). In the past ten years, the TME has received a lot of attention due to advancements in technology and growing evidence linking the TME to the emergence of cancer and treatment resistance. The tumor microenvironment (TME), a complex ecosystem that surrounds the tumor, serves as the tumor's supportive environment. Both cellular and molecular components make up TME. Immune cells, endothelial cells, and stromal cells make up the majority of TME's cellular components, while signaling molecules and extracellular matrix (ECM) proteins make up the majority of its molecular components. The immunological component encompasses all immune cells, both resident and infiltrating, including T cells, B cells, macrophages, and other immune cells. The support niches that are necessary for tumor growth are created by the stromal component, and the vascular component provides the infrastructure that transports the chemicals that are necessary for tumor cell survival. Carcinogenesis, progression, metastasis, and treatment outcomes may be improved by comprehending the TME's properties and interactions with tumor cells. For example, the vascular endothelial cells in the TME release angiogenic factors, which help tumors get oxygen and nutrients they need and encourage the formation of blood vessels. In the TME, fibroblasts and stromal cells secrete matrix metalloproteinases (MMPs), which aid in tumor invasion and metastasis. As a result of these discoveries, new pharmacological targets and drug development avenues have emerged, indicating that focusing on the TME offers effective therapeutic approaches for enhancing cancer treatment outcomes. The TME's significance in therapeutic target research and development has become increasingly apparent in recent years. 6. Focusing on the TME and its remarkable immunological cells, for instance, has revolutionized cancer treatment. The successful discovery of immune checkpoint inhibitors (ICIs), such as inhibitors of cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed cell death ligand 1 (PD-L1), and programmed cell death 1, marked a significant turning point in the treatment of cancer. The prognosis of patients with advanced cancers has been improved by these ICIs' impressive efficacy in treating a variety of solid tumors and hematological malignancies. Because TME controls treatment/drug efficacy, responsiveness, and resistance, its significance in drug development is further emphasized (Wood et al., 2014; Neophytou et al., 2021; Ganesh & Massagué, 2021; Bejarano et al., 2021; Roma-Rodrigues et al., 2019; Zhou et al., 2022).

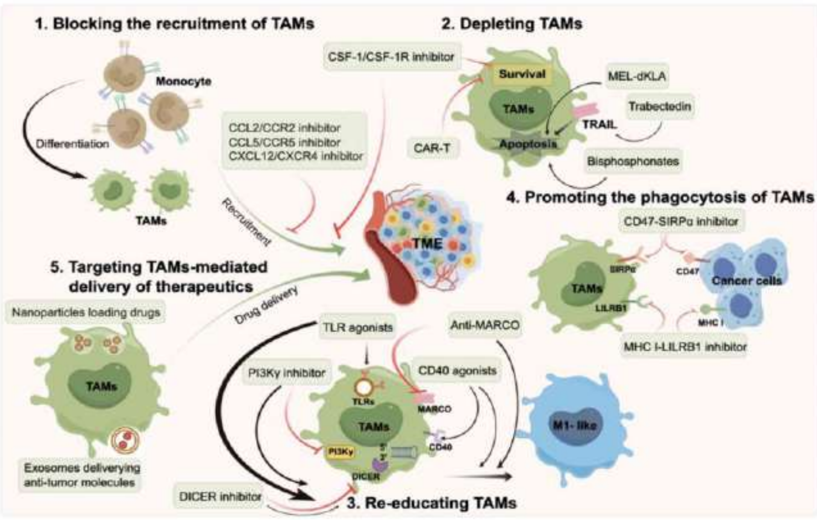

Figure 5: Therapeutic Strategies Targeting TME

It is now widely acknowledged that TME components significantly influence drug absorption, distribution, metabolism, and, ultimately, response. Immune cells in the TME can decrease the efficacy of immunotherapy and patients' responsiveness to ICIs, and the TME can restrict the administration of chemotherapy medications, which leads to drug resistance. In addition, more and more people are realizing that the TME can be used as a biomarker to predict how well a treatment will work.

6. ROLE OF TME IN CANCER PROGRESSION AND METASTASIS

Through a series of events known as metastasis, tumors move from their original site to a secondary one in order to form autonomous colonies, ultimately resulting in the patient's death. Metastasis is responsible for around 90% of cancer-associated morbidity, which also makes cancer treatment challenging and inefficient. Many factors contribute to the facilitation of metastasis. By breaking down the extracellular matrix around the tumor, matrix metalloproteinases (MMPs), which are important participants in the metastatic cascade, aid in the tumor cells' escape from the original location.

The zinc-dependent serine protease families known as matrix metalloproteinases play a role in the invasion, migration, and metastasis of tumors. Numerous cancers have been found to overexpress MMP-9, a well-known MMP linked to metastasis. Patients with higher MMP-9 expression levels had a worse outcome. Various investigations have indicated the significance of MMP-9's function in promoting metastasis. Serum MMP-9 levels were significantly higher in patients with various stages of colorectal cancer than in healthy people. Patients in the fourth stage of cancer had the greatest expression levels of MMP-9. In a similar vein, malignant brain tumors were found to express more MMP-9 than normal brain tissues. MMP-9 is a key target in anti-metastatic therapy because of its crucial involvement in causing metastases. According to several studies, metastasis can be prevented by specifically targeting MMP-9. When MMP-9 was inhibited by ribozyme in a rat sarcoma model, its capacity to spread was totally eliminated without compromising its tumorigenicity. Similarly, decreased tumor development, angiogenesis, and invasion were the outcomes of down-regulating MMP-9 in breast cancer cells.

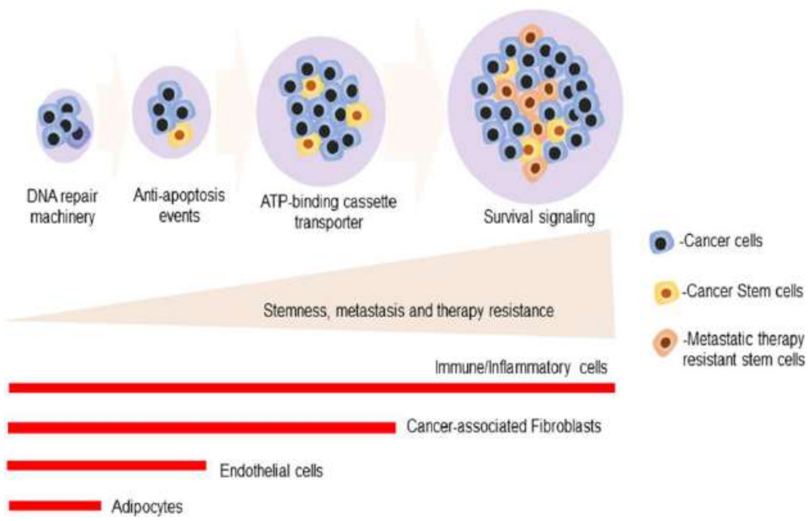

Figure 6: Role of TME

Inhibitor of KappaB Kinase beta (IKK2/IKKβ) phosphorylates and degrades Inhibitor of kappaB (IkB), hence modulating the NFθB pathway. When IkB is degraded, p50/p65 NF-B subunits are released. MMP-9 expression is down-regulated and cellular invasion occurs when the NF-B pathway is attenuated. When the NFøB pathway is activated by phorbol 12-myristate 13-acetate (PMA), MMP-9 expression rises and cell migration increases in tandem. Therefore, the NF-B pathway is a useful target to control MMP-9-mediated cancer cell invasion.

Despite the fact that metastasis is thought to be a primary cause of cancer-related death, anti-cancer therapy currently lacks an efficient therapeutic agent to target the metastatic cascade. When it comes to creating new medications to treat various illnesses, natural ingredients are always a great resource. It has been observed that a number of substances derived from natural resources suppress MMP-9 expression, hence preventing metastasis. One well-researched flavonoid, quercetin, has been demonstrated to suppress MMP-9 production, which lowers the ability of cancer cells to migrate. To find strong inhibitors of MMP-9 expression, seven less researched quercetin analogues were examined in this investigation.

7. TUMOR MICROENVIRONMENT (TME) COMPOSITION AND FUNCTION

This study highlights the roles of TGF-β and VEGF signaling in promoting angiogenesis and extracellular matrix remodeling, facilitating metastasis, and investigates how metabolic reprogramming of tumor and stromal cells drives cancer growth, influenced by nutrient availability in the TME. The study also assesses treatment strategies targeting these interactions to prevent metastasis (Tseng et al., 2018; Hansen et al., 2016).

Recent clinical findings have led to a greater understanding of the mysterious connection between the TME and cancer spread. The influence on clinical procedures and treatment development has grown as we learn more about the complexities of molecules. Treatment approaches have been transformed by the recognition of the TME as a critical component of metastasis. Novel strategies have sought to disrupt the TME rather than just target cancer cells. Due to this change, medications that modify the TME have emerged, such as immune checkpoint inhibitors and procedures aimed at restoring normalcy to tumor blood vessels.

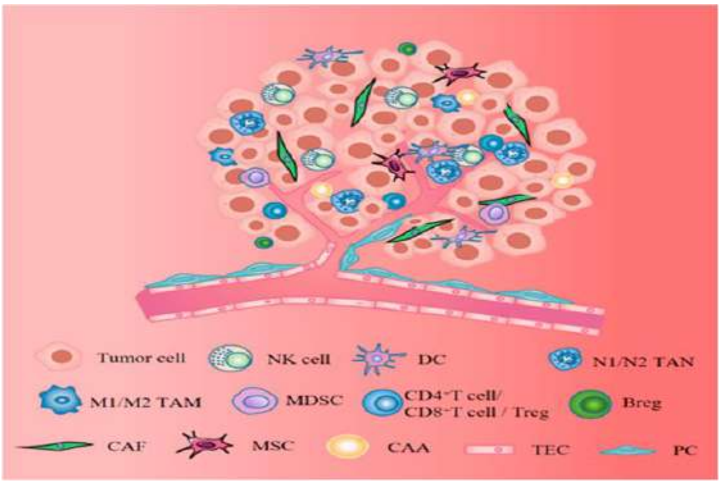

Figure 7: TME Composition and Function

A complex system, the TME is essential to the treatment of cancer. Better patient outcomes and the creation of targeted medicines are possible outcomes of this complex interaction. Future research should leverage single-cell technologies and spatial transcriptomics to elucidate the complex heterogeneity within the tumor microenvironment (TME), pinpointing the unique roles of diverse stromal components and deciphering the intricate cellular and molecular pathways that govern TME dynamics, ultimately informing the development of more targeted and effective cancer therapies. Although immunotherapy is already a game-changing treatment in oncology, each patient responds differently to it. In order to create more potent immunomodulatory treatments, future studies should focus on comprehending how cancer cells and the immune system interact within the TME. This might entail creating biomarkers that forecast immunotherapy response or resistance, allowing for individualized treatment regimens. Another promising topic is metabolic reprogramming within the TME. Comprehending these metabolic changes may aid in the creation of medications that disrupt the tumor's energy source or limit its metabolic adaptability, which is especially useful in addressing cancer cells that have spread to other locations. Research on how microbes, cancer cells, and the immune system interact could also identify novel therapeutic targets or improve on current therapies. The ethical, legal, and societal ramifications of TME research must also be continuously addressed. To guarantee that the advantages of TME research are realized in an ethical and fair manner, and treatments.

Currently, cancer therapy primarily focuses on surgical removal of the tumour followed by chemotherapy in most cases of breast cancer. But, patients with advanced stage of breast cancer are associated with poor prognosis mainly due to relapse of disease after the standard surgical treatment. Hence, a better understanding of the molecular mechanisms involved in metastasis of breast cancer is needed to control the disease. Natural compounds and many synthetic derivatives contain a wide range of substances which find its uses in treating various diseases. Thus, research has developed in investigating and synthesizing the potential anti-cancer compounds. Phytochemicals of plants have a good history of use in the treatment of cancer. According to a report more than 50% of available anticancer drugs are derived from plant sources. Currently, chemotherapeutic agents for breast cancer such as doxorubicin, paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, podophyllotoxin and its derived compounds are in use (Shabani, 2016). However, they are associated with adverse side effects and in most cases the effect is not long lasting because resistance to chemotherapy is a major obstacle for effective treatment. Recent developments in targeted therapy have shown advantages when compared to conventional chemotherapy, which have high toxicity. Anti-angiogenic therapy is one such strategy in cancer therapeutics which targets angiogenesis-related signalling molecules, including vascular endothelial growth factor (VEGF), have significantly improved the clinical outcome of cancer patients. As there is no indication for endocrine therapy or any clinically proven effective targeted therapy available for TNBC, novel and alternative therapeutic strategies are crucially needed.

8. CONCLUSION

The ethyl acetate and acetone fractions of D. bulbifera roots were shown to be a potentially rich source of bio-active chemicals that might be employed in combinatorial therapies for the treatment of breast cancer after screening for plant extracts with anti-angiogenic and pro-apoptotic qualities. Benzisoxazole derivative (7e) was one of the chemically produced compounds that shown pro-apoptotic, anti-angiogenic, and anti-proliferative properties in MDA-MB-231 and mouse mammary cancer cells. According to the SAR investigations, the biological activity was significantly impacted by the substitution position on the phenyl ring and compounds having electron-donating moiety, such as methoxy. Additionally, this is the first research demonstrating that benzoisoxazole compounds suppress MTA1-induced angiogenesis, migration, and proliferation. Compound 7e is a desirable therapeutic candidate due to its documented biological action. Using the cloning vector pGEX-5X-2-MTA1, a recombinant MTA1 protein of 80 K Da was generated, and GST-tag affinity column chromatography was used to purify it. A different new benzisothiazole derivative (5c) successfully stopped the growth of tumors caused by MTA1. At nanomolar concentrations, it was recognized as a strong anti-cancer lead structure. Particular changes in the MAPK signaling and VEGF-R2-mediated EMT pathways are ascribed to compound 5c's anti-angiogenic properties. This is the first mechanistic proof that the benzisothiazole derivative (5c) targets breast cancer mediated by MTA1. molecule 5c is acknowledged to show significant potential as a lead molecule for the treatment of breast cancer, as seen by the results above. Another tactic was to use RNA interference technology to silence the MTA1 gene. Stable transfected clones harboring MTA1-shRNA were then created, and the results were confirmed using the migration test and real-time qPCR analysis.

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Chapter-II

MOLECULAR MECHANISMS OF ANTIBIOTIC RESISTANCE IN BACTERIA

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Abstract--- The biggest and most significant hazard to human treatment of infectious diseases is antibiotic resistance in bacterial pathogens. It raises the morbidity and mortality rate, also compromises the therapeutic potential and increases the expense of successful therapy. This hazard is mostly caused by rising industrialization, significant population mobility, and the use of antibiotics. Bacteria can develop drug resistance by spontaneous mutations or the transfer of antibiotic-resistant genes (ARG) from one species to another. It is mostly caused by the long-term selective pressure that the sensitive ones apply in congested areas like blood or water bodies. Mobile genetic elements like integrons, transposons, bacteriophages, and plasmids may be the means of this transfer. There is growing evidence in recent reports that phages mobilize ARGs by generalized transduction and bestow resistance. The topic of whether horizontal transfer can be one of the key ways that drug resistance is spread at the environmental level and whether it can result in zoonotic transmission was brought up by a larger percentage of phages in environmental samples that carried antibiotic resistance genes. The problem has a significant influence on public health; hence risk analysis and containment strategies should be thoroughly studied.

Keywords--- Bacteria, Resistance, Antimicrobial, Antibiotic Resistance.

1. INTRODUCTION

The time needed to identify the pathogen has a direct impact on the diagnosis of the disease (Aswathy et al., 2021). Traditional culture-based techniques take longer, ranging from hours to days, and necessitate more biochemical and serological testing, which prolongs the time needed to characterize the pathogens and delays patient treatment. The possibility of false-positive results is a drawback of serological techniques, and certain infections lack distinct epitopes that would allow for quick diagnosis. The diagnosis of disease has been transformed by molecular diagnostic tools. DNA-based diagnostics, a new and creative technique for quickly identifying all human, animal, and plant diseases, have revolutionized the health and life science domains during the past 20 years. In addition, according to a recent study, antibiotic resistance will cost the global economy up to $100 trillion by 2050 and cause nearly 300 million deaths that could have been avoided. This problem is made worse by the fact that there isn't a strong pipeline for antibiotics (Sharma et al., 2016). This means that diseases become almost impossible to treat and doctors don't have reliable alternatives for treating infected patients. A few key ideas need be discussed in order to understand the issue of antibiotic resistance. First, because so many organisms interact with their surroundings, antimicrobial resistance has existed for a long time and is considered natural. Native bacteria have developed defenses against the majority of antimicrobial chemicals because they are molecules that are found naturally. Because of this, it's commonly assumed that these organisms are inherently resistant to one or more antibiotics.

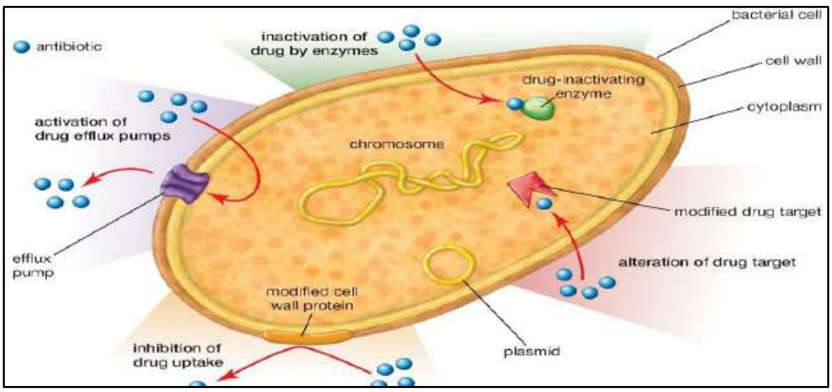

Figure 1: Antibiotic Resistance in Bacteria

Similarly, different penicillin breakpoints have been determined for Streptococcus pneumoniae depending on the levels of the antibiotic that actually enter the cerebral fluid and whether the isolate is causing meningitis or other diseases. Furthermore, Staphylococcus aureus infections treated with specific cephalosporins have demonstrated that the size of the bacterial inoculum may influence an organism's in vivo susceptibility to a given antibiotic. Evidence suggests that when cephalosporin-susceptible S. aureus causes deep-seated infections with significant inocula, some cephalosporins, such cefazolin, may not be effective.

2. GENETIC BASIS OF ANTIMICROBIAL RESISTANCE

The antibiotic "attack" is met by bacteria using two primary genetic mechanisms, according to evolutionary theory (Peterson & Kaur, 2018).

(i) Horizontal gene transfer (HGT) to obtain foreign DNA encoding resistance determinants and
(ii) gene changes that are commonly connected to the compound's mode of action.
(i) the hazardous chemical is extruded by activating efflux systems, or
(ii) Modifications to key metabolic pathways globally via regulatory network regulation.

Consequently, a variety of complex resistance is caused by acquired mutational alterations. In this part, we shall provide multiple examples of antimicrobial resistance caused by mutational alterations.

HGT

There is compelling evidence that antibiotic resistance genes in therapeutically significant bacteria are largely derived from this type of "environmental resistome". Bacteria that coexist with these substances in their environment have inherent genetic indicators of resistance. Antibiotic resistance, which impacts a variety of widely used antibiotics, has also been connected to this genetic exchange.

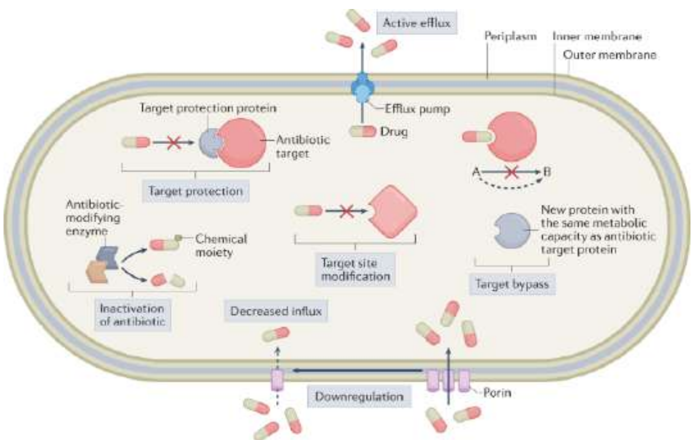

Figure 2: Molecular Mechanism

3. MECHANISTIC BASIS OF ANTIMICROBIAL RESISTANCE

Fluoroquinolone resistance, for instance, may develop via three distinct metabolic pathways (Capita & Alonso-Calleja, 2013). Gram-positive organisms, on the other hand, do not have this benefit of "compartmentalization," even though their -lactamase synthesis seems to be effective in some situations. Staphylococcal penicillinase, for instance.

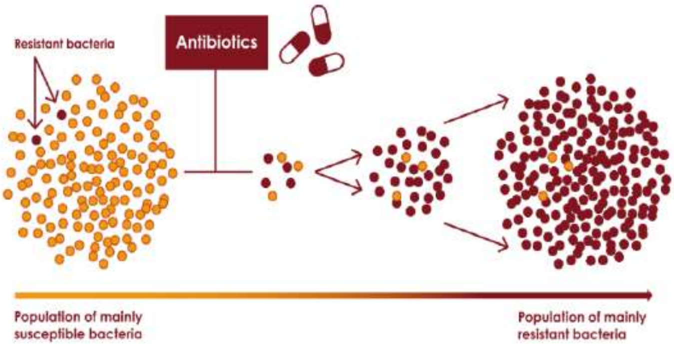

Figure 3: Natural Selection of Resistant of Bacteria

The chapter's reminder will provide a detailed explanation of the specific biochemical processes that are part of each of these mechanistic methods. Even though these enzymes are typically found in MGEs, aminoglycoside acetyltransferases from Providencia stuartii, Serratia marcescens, and Enterococcus fecium have been found to have AME-coding genes on their chromosomes. Examples of the metabolic activity of the various AMEs include phosphotransferase [APH], adenyltransferase [ANT], and acetyltransferase [ACC]. A number between 1 and 6 that represents the particular carbon on the sugar ring designates the alteration location. The first or second sugar ring is where the reaction takes place when there is a single or double prime sign.

Table 1: List of Primer Sequences Used for Species Identification

S. No	Bacteria	Gene	Primer Sequence	Product length
1	*E.coli*	*uidA*	FP 5′ TGATTTCCGTGCGTCTGAATG 3′	115bp
			RP 5′ ATGCTGCCGTAGCGTGTTTC 3′	
2	*S.aureus*	*fem B*	FP 5’ TTACAGAGTTAACTGTTACC3’	651 bp
			RP 5’ ATACAAATCCAGCACGCTCT3’	
3	*P.aeruginosa*	*PA-SS*	FP 5’GGGGGATCTTCGGACCTCA 3’	956 bp
			RP 5’TCCTTAGAGTGCCCACCCG 3’	
4	*K. pneumoniae*	BlaSHV	FP 5’GCTGGCGGTACACGCCAGCCCG 3’	995bp
			RP 5’ AGAAGCATCCTGCTGTGCG 3’	

Target Protection

Numerous drugs, including as tetracyclines (Tet[M] and Tet[O]), fluoroquinolones (Qnr), fusidic acid (FusB and FusC), and others, are affected by this mechanism. Furthermore, because this contact modifies the ribosomal conformation, the antibiotic is unable to rebind. It has also been shown that TetO changes the shape of the antibiotic's binding site and competes with Tetracycline for the same ribosomal region. Protein synthesis can proceed as a result of the molecule being forced off the ribosome.

Mechanisms of Resistance to AMPs

The morphological and functional characteristics of bacteria that AMPs come into contact with while exposed to the bacterial environment, their interactions with and passage through bacterial envelopes, and their eventual arrival at the cytoplasmic membrane or other intracellular targets are the main topics of this study on the mechanisms of AMP resistance. To get to their targets, AMPs must interact with and pass through the surrounding structures, which might differ in chemical and physical characteristics among bacteria. Understanding these interactions is essential for determining the efficacy of AMPs and creating plans to combat bacterial resistance.

With trillions of bacterial species flourishing in a wide range of habitats and environmental conditions, the bacterial kingdom is well-known for its environmental ubiquity. In the natural world, bacteria face a variety of stressors, depending on their niche. Free-living bacterial species may detect stress from changes in pH, osmolarity, or oxygen tension, whereas pathogenic bacteria experience stress from bile, antibiotics, toxins, and reactive oxygen species. Antibiotics, toxins, and ROS stress in host organisms and the environment are all examples of stress conditions that bacteria undoubtedly encounter throughout their lifespan. Other examples of stress conditions include nutrient starvation, pH and osmolarity changes, heat and osmolarity changes in industrial waste, bile stress in human guts and sewage, pH and osmolarity-related stress in various water bodies, soils, and drainage systems, and bile stress in human guts In order to deal with these stressful circumstances, bacteria employ a variety of strategies, which not only increases their likelihood of survival but also makes them more prevalent in the environment. Some of these bacteria have evolved into the most potent pathogens by employing these strategies. Beneficial coping mechanisms have emerged in bacteria as a result of the environment's rapid change. Keeping population variability at varying levels is one strategy that has enabled bacteria to endure such stressful conditions. These adaptations aid in the survival of bacteria. Bacterial heterogeneity is the cause of genetic, gene expression, or phenotypic changes in isogenic populations under constant or fluctuating environmental conditions (Frieri et al., 2017; Giedraitienė et al., 2011; Davies, 2014; Hasan & Al-Harmoosh, 2020).

Table 2: List of Multiplex PCR Primer Sequences Used for Identification of Antibiotic-Resistant Genes

S.No	Gene	Primer Sequence	Amplicon Size
1	*mecA*	FP 5'GGCCAATACAGGAACAGCAT3' RP5'TCACCTTGTCCGTAACCTGA3'	1083 bp
2	*IMP*	FP 5'AAAGGCAGCATTTCCTCTCA3' RP 5'CAAGAGTGATGCGTCTCCAA3'	408 bp
3	*Bla CTX-M1*	FP5'ACCGTCGGTGACGATTTTAG3' RP 5'CGTCACGCTGTTGTTAGGAA3'	823 bp
4	*NDM-1*	FP 5'CATTAGCCGCTGCATTGAT3' RP5'TAGTGCTCAGTGTCGGCATC3'	640 bp
5	*16SrRNA*	FP 5'TCCCGTTGAAGAGTCAAAAGA3' RP5'CGATCAATGCGGACATACAC3'	1426 bp

The simultaneous presence of two or more subpopulations from an isogenic culture with distinct genetic or phenotypic characteristics is known as population heterogeneity. The stochastic behavior of transcription, in which the promoter region is bound by transcription factors or promoter binding elements through random molecular encounters, is the cause of inherent stochasticity. Only a small number of these molecules may be present in the cell, and even then, their quantities may be extremely small. Gene expression may become more stochastic as a result of this alone. These kinds of fluctuations are referred to as intrinsic fluctuations. The deactivation of the promoter, the stability and decay of mRNA, protein production, and the turnover or decay of protein are all examples of intrinsic fluctuations. In addition to these intrinsic fluctuations, extrinsic fluctuations contribute to the stochastic behavior of bacteria or cells. Variables in the cell that have an indirect effect on transcription or translation within the cell are the source of extrinsic fluctuation, or noise. As a result, the cell's intrinsic and external fluctuations contribute to the overall stochasticity. In addition to bet hedging, there is a theory of self-destructive cooperation. In the population, there are two phenotypic variations of this adaptive trait; only one of them can reproduce, while the other dies. In greater detail, a subpopulation with a distinct phenotype makes sacrifices in order to improve the majority of the population and ensure its survival.

Table 3: List of MLST Primer Sequences Used for Sequence Typing of Staphylococcus Aureus Isolates

S.No	Gene	Primer Sequence	Amplicon Size
1	arc	FP 5' TTG ATT CAC CAG CGC GTA TTG TC -3' RP 5' AGG TAT CTG CTT CAA TCA GCG -3	456 bp
2	aro	FP 5' ATC GGA AAT CCT ATT TCA CAT TC -3' RP 5' GGT GTT GTA TTA ATA ACG ATA TC -3'	456 bp
3	glp	FP 5' CTA GGA ACT GCA ATC TTA ATC C -3' RP 5' TGG TAA AAT CGC ATG TCC AAT TC -3'	465 bp
4	gmk	FP 5' ATC GTT TTA TCG GGA CCA TC -3' RP 5' TCA TTA ACT ACA ACG TAA TCG TA -3'	417bp
5	pta	FP 5' GTT AAA ATC GTA TTA CCT GAA GG -3' RP 5' GAC CCT TTT GTT GAA AAG CTT AA -3'	474bp
6	tpi	FP 5' TCG TTC ATT CTG AAC GTC GTG AA -3' RP 5' TTT GCA CCT TCT AAC AAT TGT AC -3'	402bp
7	yqi	FP 5' CAG CAT ACA GGA CAC CTA TTG GC -3' RP 5' CGT TGA GGA ATC GAT ACT GGA AC -3'	516 bp

For example, bacteria lyse to release a chemical that helps the remaining cells survive. These phenotypic variations are the cause of the public good. When bacterial toxins are released, the same thing happens: the cells that make the toxins lyse and kill other microbiota while helping their own species survive. In order to increase population survival in various bacterial systems, the aforementioned examples demonstrated heterogeneity in a variety of ways. Phenotypic diversity is also maintained in mycobacterial populations, according to recent research. Understanding the significance of Mycobacterium tuberculosis as a pathogen and the challenges it presents in terms of medication resistance is essential before delving into the specifics of mycobacterial heterogeneity. In the mycobacterial population, asymmetric cell division results in both normal/long and short cells. Even though short mother cells divide symmetrically, the feeder line for the production of short cells still uses asymmetric cell division. Two distinct subpopulations of mycobacteria have emerged from mycobacterial cultures, each with varying degrees of antibiotic resistance and mutability. The asymmetrical partitioning of these components during ACD may be the cause of the asymmetrical or asymmetrical expression of NADH oxidase, which results in the distinct production of ROS in short cells as opposed to normal cells. The antibiotic is expelled via a pump from the bacterial cell. Pumps that are housed inside bacterial cell walls or membranes can be created. These so-called efflux pumps are found in many bacteria and can move a variety of materials, such as nutrients and signal molecules. Several of these pumps can also move antibiotics out of the bacterium to lower the concentration of antibiotics in the cell. When bacteria manufacture more of a certain pump due to DNA mutations, resistance may increase. decrease the permeability of the membrane enclosing the bacterial cell. The bacterial membrane goes through some changes that make it harder to get through. Because of this, less antibiotic reaches the bacteria. Get rid of the antibiotic. Certain bacterial enzymes have the potential to render antibiotics ineffective. One such is -lactamase, which breaks down the -lactam ring, the active component of penicillins, which are vital medications used to treat infections in humans. In recent years, bacteria that manufacture extended-spectrum beta-lactamases, or ESBLs, have grown to be a significant problem. A range of beta-lactam antibiotics, even those used as a last option to treat bacterial infections, can be broken down by them (Martinez, 2014).

Modify the Antibiotic

Enzymes that can add different chemical groups to antibiotics are infrequently produced by bacteria. Consequently, the antibiotic and its bacterial cell target do not form a connection.

Camouflage the Target

If the bacterium's target undergoes structural or compositional changes as a result of bacterial DNA mutations, the antibiotic may no longer interact with the target. on protect the target structure from the antibiotic, the bacteria can instead affix a range of chemical groups on it. Different proteins can be expressed. Certain bacteria are capable of producing alternative proteins that can be used in place of the ones that the antibiotic inhibits. For instance, Staphylococcus aureus is capable of producing a novel protein that binds penicillin and acquiring the resistance gene mecA. Beta-lactam antibiotics target these proteins, which are necessary for the formation of bacterial cell walls. The novel penicillin-binding protein's low affinity for -lactam antibiotics allows the bacteria to resist treatment. This kind of resistance is the foundation of methicillin-resistant Staphylococcus aureus, or MRSA.

Reprogram Target

Sometimes bacteria can create a new version of a structure they need. For example, the cell wall produced by vancomycin-resistant bacteria differs from that of vulnerable bacteria. This kind of cell wall does not interact with the antibiotic as well. Certain microorganisms have innate resistance to specific antibiotics. Consider, for instance, an antibiotic that breaks down the bacterial cell wall. An antibiotic won't work on a bacterium if it lacks a cell wall. We refer to this phenomenon as intrinsic resistance. Acquired resistance is the term used to describe the development of resistance in a bacterium that was previously susceptible to an antibiotic.

Table 4: Gram Negative Bacteria Identification

Bacteria	**MacConkey agar**	**Oxidase**	**Indole**	**MR**	**VP**	**Citrate**	**H_2S**	**Urease**	**Motility**
Escherichia coli	LF	-	+	+	-	-	-	-	+
Klebsiella pneumoniae	LF	-	-	-	+	+	-	+	-
Pseudomonas aeruginosa	NLF	+	-	-	-	-	-	-	+

4. STRATEGIES TO COMBAT ANTIBIOTIC RESISTANCE

Salmonella is a complex Gram-negative bacterium that is the leading cause of illness worldwide. According to the WHO, salmonella is among the fourth most common causes of diarrheal disease globally. One of the microorganisms that could acquire antibiotic resistance and evade the toxicity of antibiotics is Salmonella species, which is a global health problem. In-depth understanding of the molecular processes and underlying metabolic changes thought to be responsible for Salmonella's antibiotic resistance is the goal of this review. This knowledge will support cutting-edge research into creating new, more effective medications and aid in understanding and lessening the effects of antibiotic-resistant bacteria on people (Urban-Chmiel et al., 2022).

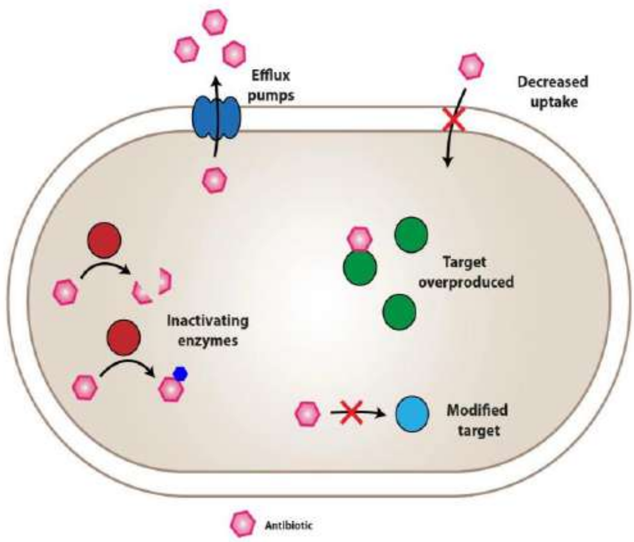

Figure 4: Antibiotic Resistance Spread

Since a large percentage of yearly deaths are caused by illnesses that are resistant to treatment, antimicrobial resistance (AMR) is a serious global health concern. Antimicrobial peptides (AMPs) have shown promise as substitutes for traditional antibiotics (ATBs) during this crisis. These cationic peptides, which are naturally produced by all kingdoms of life and have broad-spectrum activity against bacteria, fungi, viruses, and parasites, are essential for the innate immune system of multicellular animals as well as bacterial interspecies competition. AMPs primarily cause cell lysis by breaking down the membranes of bacterial pathogens, though they use a number of different tactics. However, a gradual co-evolution between microbes and their hosts has led to the emergence of bacterial resistance to host AMPs. In order to expand and improve these processes' potential for creating and using them as treatments for MDR bacteria, we also investigate their selectivity toward structurally varied AMPs. Furthermore, we shed light on the importance of AMP resistance in relation to host-pathogen interactions.

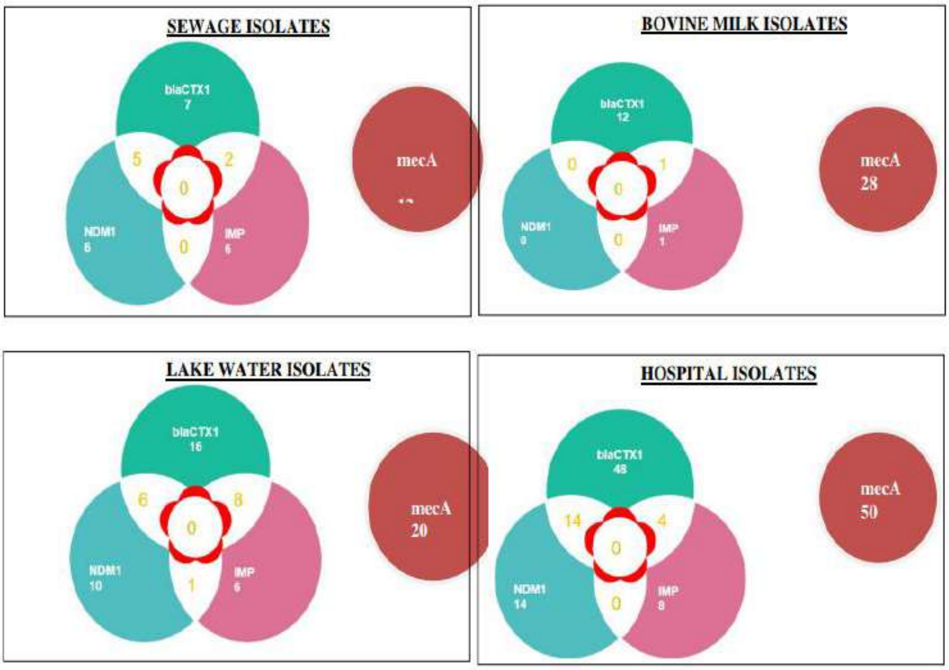

Figure 5: Venn Diagram Depicting the Number of Isolates Having Antibiotic Resistant Genes

Aside from the non-host peptides polymyxins, bacteria have become resistant to AMPs, albeit this phenomenon is thought to be less common at the moment and develops more slowly than with conventional antibiotics. Constitutive (passive and intrinsic) resistance to AMPs can take the form of mechanisms derived from an organism's innate characteristics that provide resistance even when AMPs are not present (Jian et al., 2021; Biswas et al., 2019; Baylay et al., 2019). These characteristics, like the elements of bacterial cell walls and membranes, may be crucial to the survival of the bacteria. Certain bacteria have intrinsic resistance to polymyxins in vitro, including the Gram-negative species Burkholderia cepacia complex (BCC), Morganella morganii, Proteus spp., Providencia spp., Serratia spp., Edwardsiella tarda, and Neisseria spp. The primary cause of this resistance is the constitutive production of lipopolysaccharides (LPSs) that have been electrostatically rejected by positively charged 4-amino-4-deoxy-L-arabinose (L-Ara4N). Since only conditional mutants in this pathway remain alive, it has been demonstrated that these modifications are necessary for Burkholderia cenocepacia survival.

5. MECHANISMS OF ANTIBIOTIC RESISTANCE

The spread of microorganisms resistant to certain antibiotics jeopardizes the ability to treat infections worldwide. Despite the fact that it is typically attributed to chromosomal changes, resistance is most frequently linked to extrachromosomal components obtained from other bacteria in the environment. These include integrons, transposons, and plasmids, among other types of mobile DNA segments. It is now known that intrinsic processes, such as efflux pumps, which expel a variety of antibiotics, are primarily responsible for bacteria's multidrug resistance. Multidrug-resistant organisms persist and multiply worldwide, resulting in public health emergencies and clinical failures in illness treatment. Bacteria develop intricate defense mechanisms to ward off the lethal effects of antibiotics (Darby et al., 2023).

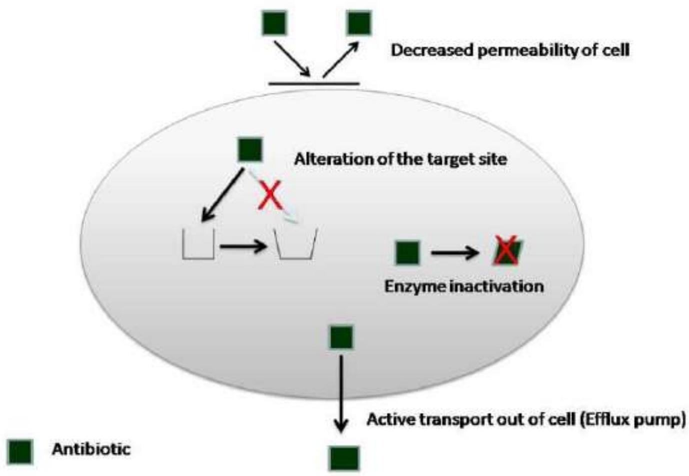

Figure 6: Mechanisms of Antibiotic Resistance

New medications can be made easier with the knowledge of these resistance pathways. As we address this pressing issue, important considerations include the mobility of the microbial genome and the relative ease with which resistance might develop through gene acquisition or mutation. The creation of novel medicines, which are sorely needed to treat infectious diseases, ought to rekindle admiration given the possibility of resistance. Globally, antimicrobial agent resistance has become a leading cause of illness and mortality. When antibiotics were first developed in the 1900s, we thought we had defeated microbes. But it was soon found that the bacteria may become resistant to any therapy. Most pathogenic microorganisms seem to be able to become resistant to at least some antibacterial substances.

Table 5: Antibiotic Resistant Pattern

Antibiotics	**Clinical Samples**	**Bovine Milk samples**				**Sewage Water samples**
	Chettinad Hospital N=100	**Kelambakkam N=25**	**Sholinganallur N=25**	**Perungudi N=25**	**Nellikuppam N=25**	**Sewage Water Treatment Plant, PerungudiN=12**
No of E. coli +ve isolates	92	9	10	9	8	12
Piperacillin-Tazobactam	58	2	4	3	1	11
Imipenem	44	2	1	1	0	10
Cefepime	60	2	4	4	1	11
Cotrimoxazole	60	2	4	4	1	11
Amikacin	58	3	3	3	1	10
Ciprofloxacin	54	2	3	3	1	11
Multi-Resistance +++	60/92	3/9	4/10	4/9	1/8	11/12
% of Resistance	60/92 (65%)	12/36 (33%)				11/12 (92%)

Drug inactivation, drug target modification, drug uptake restriction, and drug efflux are the main mechanisms of resistance. Improved treatment choices for infectious diseases and antimicrobial drugs that can withstand the bacteria's resistance-building efforts should result from a deeper comprehension of these mechanisms. These mechanisms may have been acquired by the microorganisms from other microbes or they may have originated naturally. The medical community thought the battle against infectious diseases was won when antibiotics were discovered. But now that many of the bacteria have become resistant to a variety of antimicrobial medicines, the fight seems to have switched in the bacterium's favor. Infectious diseases now contribute significantly to global morbidity and mortality. According to the World Health Organization (WHO), lower respiratory infections, diarrhea, malaria, and HIV/AIDS rank among the top 10 causes of morbidity and mortality. Antimicrobial resistance has led to large increases in the effect of infectious diseases, the number of infections, and the expense of healthcare provision. Every antimicrobial treatment we use to treat illnesses has bacterial resistance, which is well known to exist. When a new drug is approved for usage, this resistance spreads quickly. In response to these concerns, the World Health Organization (WHO) published a Global Action Plan on Antimicrobial Resistance in 2015. The classification of antimicrobial agents is made possible by the mechanism of antimicrobial activity. The main categories include compounds that inhibit the production of proteins and nucleic acids, depolarize the cell membrane, stop bacterial metabolism, and stop bacterial cell walls from forming. gives instances of drugs from each of these groups. With so many distinct systems, we ought to be able to better govern the organisms. Sadly, poor stewardship of antimicrobial medicines is a part of the massive resistance problem we face today. Increased usage of antimicrobial drugs by both humans and animals, as well as prescriptions for treatment that is inappropriate for the bacteria in question, are some of the factors that have contributed to the problem of resistance. Some well-known antimicrobials may be overused by doctors because they choose them for their low cost and low toxicity. Additionally, prescription errors may occur for antimicrobial medications. For instance, a broad-spectrum medication may initially be prescribed but later found to be ineffective against the infection-causing organisms.

Table 6: Comparison of Drug Resistance by Antibiogram and Uniplex PCR Results of Bovine Milk Isolates

Place of collection	**Organisms isolated**	**Antibiotic Sensitivity**			**PCR results (No of +ve)**			
		R	**S**	**Grand Total**	**mecA**	**IMP**	**Bla-CTXM1**	**NDM 1**
Nellikuppam								
	E.coli	1	7	8	0	0	1	0
	K.pneumoniae	0	1	1	0	0	0	0
	P.aeruginosa	0	3	3	0	0	0	0
	S.aureus	4	4	8	4	0	0	0
Kelambakkam								
	E.coli	3	6	9	0	0	3	0
	K.pneumoniae	0	1	1	0	0	0	0
	P.aeruginosa	0	4	4	0	0	0	0
	S.aureus	6	4	10	6	0	0	0
Perungudi								
	E.coli	4	5	9	0	0	4	0

	K.pneumoniae	0	1	1	0	0	0	0
	P.aeruginosa	1	3	4	0	1	0	0
	S.aureus	9	9	18	9	0	0	0
Sholinganallur								
	E.coli	4	6	10	0	0	4	0
	K.pneumoniae	0	1	1	0	0	0	0
	P.aeruginosa	1	3	4	0	1	1	0
	S.aureus	9	9	18	9	0	0	0
Grand Total		**42**	**67**	**109**	**28**	**2**	**13**	**0**

The risk is that people may use antibiotics too much, which could cause organisms to develop resistance. Additionally, patients who have been exposed to antimicrobials the most frequently have resistant bacteria, and individuals who have previously taken antimicrobial medications are more likely to contract a drug-resistant organism. It's possible that different species or groups of bacteria aren't always equally vulnerable to or resistant to a particular antibiotic. Even within related bacterial groups, resistance levels might differ greatly (Xia et al., 2016).

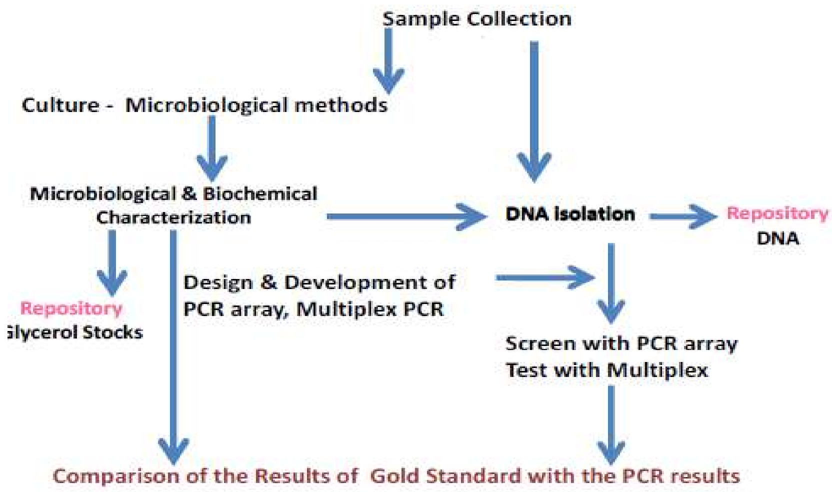

Figure 7: Flow Chart of Sampling

Tests for sensitivity and resistance frequently employ the minimum inhibitory concentration (MIC), which is the lowest concentration of a drug that will stop bacteria from growing. In actuality, an antibiotic's susceptibility is a range of the typical minimum inhibitory concentrations (MICs) for that specific antibiotic in bacteria of the same kind. If a species' average MIC falls inside the resistance range, it is considered to have inbuilt resistance to the drug. Furthermore, bacteria can obtain resistance genes from related organisms; the species and genes obtained will dictate the bacteria's resistance. Natural resistance can be classified as either induced (the genes are naturally present in the bacterium but only express to resistant levels after exposure to an antibiotic) or intrinsic (the genes are constantly expressed in the species).

6. CONCLUSION

Compared to the origin of bacterial species on our planet, the use of antimicrobials in medical treatment is relatively young. One of the most effective developments in modern medicine, the introduction of antimicrobial therapy into clinical practice, opened the door for intricate and sophisticated medical procedures that have significantly extended people's lives worldwide. In order to survive, bacteria have developed sophisticated and creative defense mechanisms against antibiotic attacks. This process is probably impacted by the growing usage of antibiotics in clinical practice. Antibiotic resistance, which has increased dramatically in recent decades, is one of the most pressing issues affecting public health in the twenty-first century. In actuality, the prevalence of infections in clinical settings that are incurable because the infecting organism is resistant to numerous treatments has increased. As big pharma focused on other, more lucrative, and fulfilling ventures, the tide of resistance rose. We must greatly increase and support research and development activities if we are to find a solution. Developing new tactics to address the problem requires an understanding of the processes by which bacteria gain antibiotic resistance. The development of antibiotics must take into account the fact that microbes will react to them and become resistant to them—this is an evolutionary fact. Research on the factors behind antibiotic development and resistance should therefore be tenacious, robust, and continuous. The "war" against organisms with a high degree of adaptability and survival is probably going to go on for a while.

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Chapter-III

THE ROLE OF INFLAMMATION IN CARDIOVASCULAR DISEASE

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Abstract--- The immune system has a major contribution to the initiation and progression of atherosclerosis, a chronic inflammatory condition. Significantly, cardiovascular diseases (CVDs), to which atherosclerosis is a major contributor, are the number one cause of death worldwide, responsible for about 31% of total deaths, and a major cause of illness globally, as per the World Health Organization (WHO). These are the world's most important health problems. Among the chronic heart conditions include arrhythmia, congestive heart failure, myocardial infraction, heart stroke, coronary artery disease, cardiac ischemia, and atrial fibrillation; early detection of these conditions may lower the death rate. Traditional diagnosis of these heart diseases is done by the physical examination of the reports related to concerned symptoms of patient with the reports of his medical history. Analysis done through these methods may not be accurate in detection of heart diseases and it also expensive and time consuming because these diseases require continuous monitoring. Low-density lipoprotein cholesterol oxidizes inside the artery wall. Furthermore, residual lipoproteins and lipoproteins high in triglycerides have pro-inflammatory properties, which is mediated by an increase in sympathetic output that releases inflammatory cytokines. Through an increase of leukocyte clones in peripheral circulation, inflammation also serves as a link between aging and cardiovascular disease Ho (2018).

Keywords--- CVDs, Anti-inflammatory, Monitoring, Myocardial.

1. INTRODUCTION

The conversion of risk factors into CVD is largely dependent on the vascular endothelium. After entering the subendothelial region, LDL-C oxidizes and aggregates into big complexes. Selective inhibitors of cytokine pathways with anti-inflammatory drugs are able to lower the risk of myocardial infarction and stroke but in the process pose an increased risk of infections. In the environment of inflammation, lipoprotein metabolism is dysregulated, which leads to the production of sub-fractions of small dense LDL-C (sdLDL-C) having decreased affinity for the liver-specific LDL-C receptor. A higher risk of CVD is linked to elevated sdLDL-C levels. This, in turn, encourages a low-grade inflammatory response in the blood vessels, which advances atherosclerosis. Inflammation is a critical pathway that connects other cardiovascular risk factors to the pathogenesis of atherosclerosis, resulting in coronary artery disease (CAD), stroke, and cerebral aneurysms. The whole atherosclerotic process, from lipoprotein deposition to plaque development and rupture, is coordinated by a highly interconnected network that includes the immune system, bone marrow, and spleen, which control the balance between pro- and anti-inflammatory reactions through cytokines, leukocytes, macrophages, and lymphocytes. The way that statins lower the risk of cardiovascular disease confirms the function that inflammation plays in atherosclerosis. According to a number of studies, statins' primary positive effects which are somewhat independent of their ability to lower cholesterol come from reducing vascular inflammation Kumar & Sinha (2020).

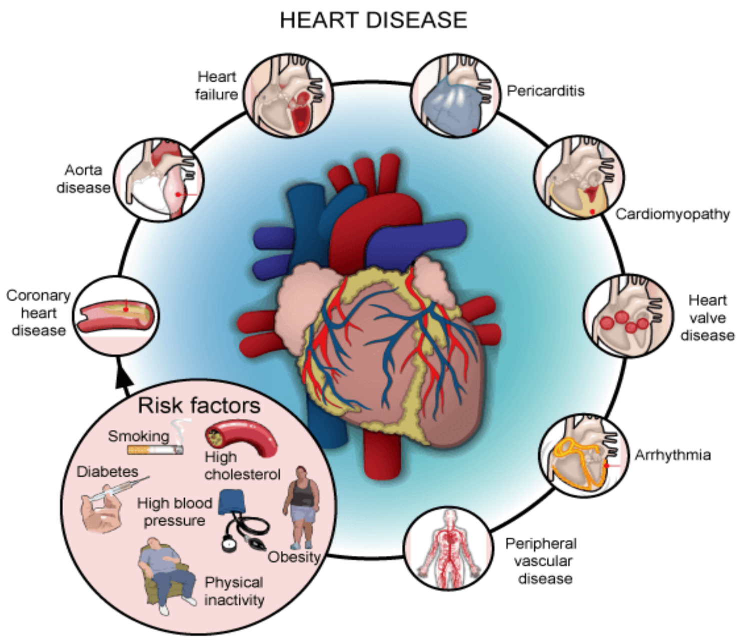

Figure 1: Cardiovascular Disease

2. THE CVD RESPONSE AND ITS ROLE

Endothelial Dysfunction

A brain aneurysm can also occur as a result of arterial remodeling brought on by WSS alterations. The plaque becomes hypoxic due to inflammatory alterations, which causes neovascularization to form from adventitial vasa vasorum. This procedure increases the susceptibility of plaque Ma et al. (2020).

Plaque Calcification

PET-based longitudinal imaging investigations have demonstrated that calcium is subsequently deposited at inflammatory arterial sites, and that the rate of calcium deposition varies within the same arterial segment depending on the degree of inflammation. These crystals can combine and embed themselves in the fibrous cap as microcalcifications that are smaller than 50 μm in diameter. Plaque calcification increases the number of nucleating sites and new calcification by further inducing macrophage infiltration. The growth of microcalcification will result from further cycles of monocyte infiltration that evolve into macrophages and then die if inflammation continues.

Furthermore, microcalcifications create a mechanical tension in the fibrous cap that exacerbates the inflammation surrounding the lesion. Death of macrophages and smooth muscle cells results in the release of vesicles, which serve as nucleating sites for hydroxyapatite crystal deposition. Biomechanical investigations indicate that plaque rupture is possible because of extreme stress at the tissue interface with varying stiffness, e.g., hard microcalcifications in the softer fibrous cap, causing a large modulus mismatch. According to this impact, the larger the interface area, the greater the chance of a plaque rupture. Microcalcifications are rare and the risk is minimal when inflammation is still in its early stages. Their number and the size of the contact between the stiff and soft zones both grow as long as inflammation continues.

Vulnerable Plaque

Although stable plaques have also been observed to have some degree of inflammation, inflammation is a crucial characteristic of fragile plaques. The extracellular matrix (ECM), which is produced by smooth muscle cells in the artery wall and includes collagen, elastin, proteoglycan, and glycosaminoglycan, makes up the majority of atherosclerotic plaques. Plaque calcification and the ECM are intertwined, and both contribute to the stability of the plaque. Microcalcification localizes between collagen fibers and collagen-deficient areas, resulting in an inverse relationship between microcalcification and ECM proportions.

1. Key Inflammatory Mediators and Pathways

Inflammation Targeted Therapy

Changes in lifestyle, a decrease in risk factors, and lipid-lowering medication are the cornerstones of CV prevention. Acute cardiovascular events are still a significant risk for people with atherosclerosis, particularly those who are older or have comorbidities, even with the best medical care and a decrease in LDL-C levels. Observational research has demonstrated that a particular anti-inflammatory drug decreases the occurrence of atherosclerosis-related complications in patients with rheumatic disease, which is typified by elevated circulating cytokine levels. Additionally, statins reduce inflammation and cholesterol, both of which help to reduce the risk of cardiovascular disease (Townsend et al., 2016; Townsend et al., 2015)

Through the mobilization of various integrin types and other molecules, adhesion molecules play an important function in the immune system. Nevertheless, little is understood about the intricate relationships that exist between adhesion molecules and other elements of the inflammatory process when atherosclerotic plaque develops Aswathy et al., (2022).

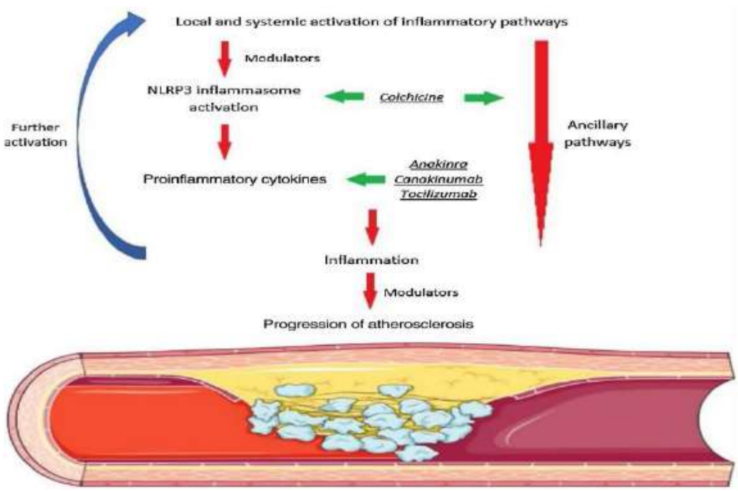

Figure 2: Inflammation in CVD

It is well established that hyperlipidemia is essential to the onset and advancement of atherosclerosis. Unresolved inflammation, which is essential for atherogenesis, may result from an imbalance in cholesterol levels. Early atheroma formation is characterized by the recruitment of many subcellular entities and the release of proinflammatory cytokines, which is an indication to exacerbate the atherosclerotic process. Prolonged inflammation exacerbates atherosclerotic plaque, which leads to myocardial infarction and thrombus development. (Tayb 2016; Libby 2002). It is possible to think of inflammation as a sequence of cellular and molecular processes that heal wounds by repairing or restoring tissue. A new humoral response for cellular growth and reformation may be produced as a result of a normal inflammatory response. When a chain reaction of inflammation fails to resolve, it eventually leads to organ damage and even death Wei-Wei et al. (2017). Reactive oxygen species, which are produced by cellular metabolism, are essential for triggering signaling pathways that change intracellular and extracellular metabolism. However, practically all of the ROS produced in the cell are produced by the mitochondrial respiratory chain. A respiratory explosion and the subsequent generation of ROS are caused by the development of an inflammatory response, which also increases the concentration of mast cells and leukocytes in the area. Along with changing inflammatory cytokines like IL-1β, TNF-α, and IL-6 and transcription factors like NF-κB and HIF1-α, the inflammatory cells can also create essential markers that initiate signal transduction cascades. Antioxidants are compounds that lower the inflammatory response by preventing the generation of reactive oxygen species. Antioxidants are very helpful in preventing oxidative damage because inflammation starts a vicious cycle.

2. Therapeutic Strategies Targeting Inflammation

Cardiovascular disease (CVD) is a major global concern and one of the primary causes of death. A key element in the pathophysiology of these illnesses is the accelerated atherosclerosis process, which has been examined in relation to inflammation as a risk factor. Several inflammatory biomarkers, such as C-reactive protein (CRP), interleukins (ILs), and tumor necrosis factor-alpha (TNF-α), have been implicated in the atherosclerotic process and are associated with systemic inflammatory diseases and cardiovascular diseases (CVDs) Bhatnagar et al., (2015). The risk of CVDs may be predicted using these markers. Knowing the exact mechanisms can help develop treatment plans that focus on pro-inflammatory processes Smith & Edelman (2023).

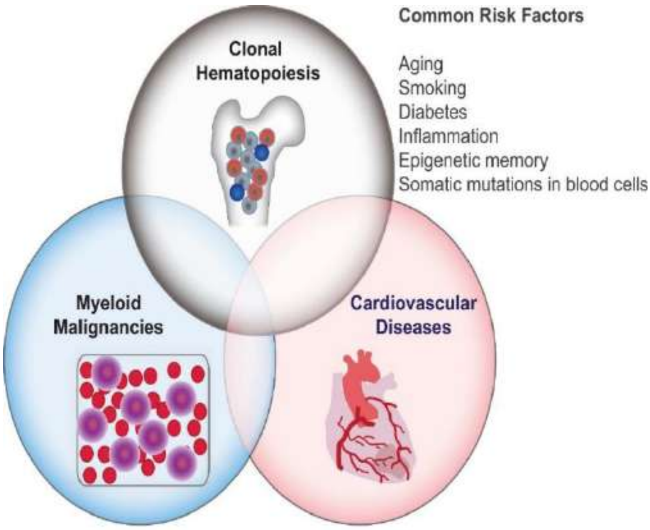

Figure 3: Clonal Hematopoiesis

Numerous families of inflammatory cytokines have been found to be responsible for endothelial dysfunction, which raises the risk of CVDs. Nonetheless, research has shown that inflammatory processes contribute to artery wall stiffness, which raises the risk of CAD. Aside from acquired risk factors including cancer, trauma, and surgery, research has also indicated that inflammation plays a part in arterial thrombosis and VTE. The degree of VTE is known to be correlated with a number of inflammatory indicators.

Novel treatments that target these inflammatory biomarkers may result from research on the function of inflammatory processes, thereby lowering the burden of CVDs. Colchicine's function in treating pericarditis and averting atrial fibrillation following heart surgery and ablative therapy has been well investigated. In addition to evaluating the various indicators and potential treatment targets, this thorough review attempts to appraise the data currently available about the involvement of inflammation in cardiovascular illnesses. Plaque instability and a high risk of rupture are caused by the decreased NO-mediated vasodilatory response and increased arterial stiffness. In order to forecast a long-term prognosis and evaluate the risk and severity of unfavorable cardiovascular events, serum levels and gene polymorphisms in a number of markers have been employed as independent determinants. Additionally, vascular function has improved as a result of the application of anti-inflammatory medication that targets these indicators in chronic inflammatory illnesses. New anti-inflammatory treatments for improved cardiovascular outcomes may result from future research on inflammatory pathways, which will provide additional insight into the biomarkers involved Wilkins et al., (2017).

3. Therapeutic Strategies Targeting Inflammation

A complicated and highly conserved series of molecular and cellular processes make up inflammation. Known as "the fire within," inflammation is tightly controlled and essential for tissue regeneration and host defense. Inflammation is generally advantageous and has developed to aid with survival. Until recently, atherosclerosis was thought to be a condition caused by passive cholesterol buildup in the subendothelial region, despite data acquired by Virchow more than a century ago suggesting inflammatory white cells contribute to atherogenesis.

MicroRNAs

Gene expression has been demonstrated to be modulated by microRNAs, which are small non-coding RNAs. In humans, over 2000 microRNAs have been identified. According to recent studies, microRNAs play a big part in controlling inflammation. The expression of microRNA by immune and bystander cells can contribute to an inflammatory feedback loop that, depending on the kind of microRNA generated, can either enhance or reduce inflammation. Numerous microRNAs that promote and inhibit inflammation have been identified. This enables the reduction of inflammation in locations that are experiencing active inflammation. Currently, one of the biggest obstacles is the expense of developing microRNA drugs and the methods of administering them.

Targeting the NLRP3 Inflammasome Pathway

Based on CIRT and CANTOR data, vascular risk is reduced by blocking the inflammasome-to-CRP pathway. Consequently, a number of research are looking directly at the function of NLRP3 inhibition. One potential benefit of directly inhibiting the inflammasome is that it may prevent inadvertent immunosuppression, which can occur when cytokines are systemically targeted. Numerous inflammasome inhibitors, both direct and indirect, are being studied.

Compared to indirect inhibitors, direct inhibitors like CY-09, MCC950, exhibit reduced off-target immunosuppression. It has been demonstrated that IL-18 levels, can predict CVD, albeit not as well as IL-1β or IL-6.

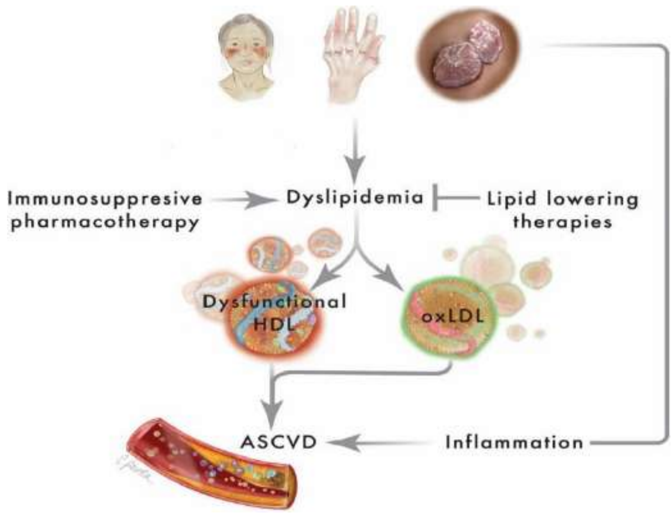

Figure 4: Inflammatory Disease

Omega-3 Fatty Acids

It's interesting to note that this positive impact happened regardless of the initial or final serum triglyceride levels. It is unclear what possible processes could be responsible for this advantage. However, there was a significant drop in hsCRP levels among those randomly assigned to icosapent ethyl compared to placebo (median percent change from baseline, −12.6% vs. 29.9%; $P < 0.001$), indicating that EPA supplementation may have an anti-inflammatory impact. A decrease in triglyceride levels is one way that EPA may have anti-inflammatory effects. EPA also serves as a precursor to the resolvins, anti-inflammatory lipid mediators that aid in the resolution of chronic inflammation. The anti-inflammatory actions of omega-3 fatty acids, such as decreased cytokine production and endothelial and platelet activation, can be responsible for their reported benefit. Additional studies are being conducted to define the precise mechanisms by which EPA modulates inflammation. (Van de Voorde et al., 2013; Cosselman et al., 2015)

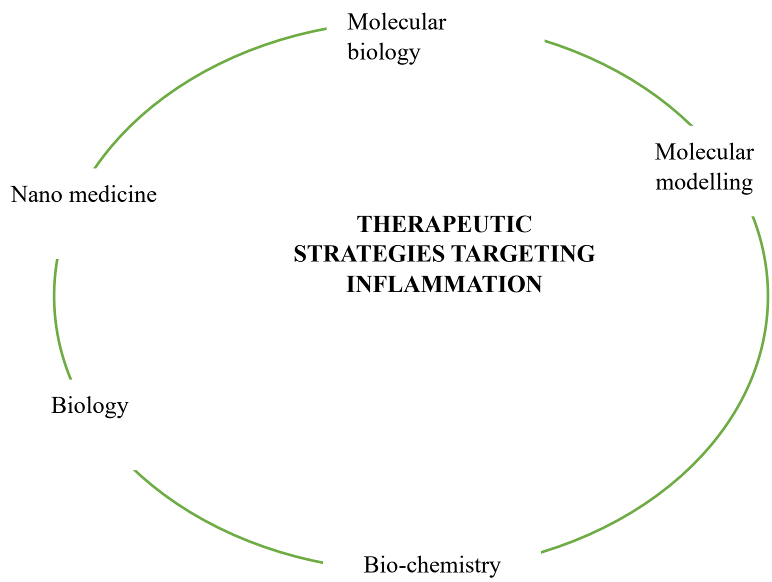

Figure 5: Therapeutic Strategies Targeting Inflammation

Microbiome Modification

The intricate relationship between the immune system, the gut, and its microbial ecology is being supported by mounting data. Alterations in the gut microbiota's makeup could put the gut at risk for inflammation, which would increase systemic inflammation. There are several levels of contact. The microbiome may be involved in regulating mucosal integrity, according to data from observational research on humans and animal models. The mucosal epithelium of the colon depends mainly on butyrate and short-chain fatty acids (SCFA) derived from the microbiome's fermentation of dietary fiber. A low-fiber diet can change epithelial metabolism, enhancing permeability and decreasing SCFA and butyrate production. This results in an inflammatory response, both locally and systemically, as a result of the transfer of bacteria and lipopolysaccharides. Studies have demonstrated that blood hsCRP levels are inversely related to bacterial genetic markers of butyrate production in the gut. Butyrate controls the local gut inflammatory response and immunologic homeostasis by altering colonic regulatory T-cells. Gut inflammatory response alterations may result in shifts in the bacterial composition, establishing a chronic low-grade inflammatory state. The microbiome also modulates the action of a Western diet by generating TMAO precursors from choline, betaine, and carnitine, which are found in red meat, eggs, fish, and poultry. (Berry et al., 2012; Lopez et al., 2023; Bansal et al., 2020)

4. FUTURE ANALYSIS

Additional prospective research is required to validate these results. Current studies are looking into strategies to control the gut's microbial ecosystem and disrupt the possible feedback loop between systemic and intestinal inflammation. Dietary intervention and the use of prebiotics are two potential pathways being studied; these strategies seek to improve epithelial integrity and favorably change the makeup of microbial communities in order to reduce inflammation systemically.

5. CURRENT SUCCESSES AND CHALLENGES

In spite of impressive therapeutic progress, cardiovascular diseases are still the predominant cause of morbidity and mortality globally. Since cardiovascular diseases tend to be inflammatory in nature, alternative treatment methods are needed. Inflammation is a normal response to tissue injury, being mediated by cytokines, chemokines, and leukocyte activation. But unchecked chronic inflammation results in tremendous tissue injury. Experimental studies point to the pivotal role of inflammation in cardiovascular disease, such as hypertension, atherosclerosis, myocardial infarction, and hypertrophic heart failure. In addition, metabolic preconditioning and cardiovascular disease have significant correlations. Inflammatory associations with disorders like diabetes and obesity have existed since the 1960s, when increased circulating fibrinogen and other acute-phase proteins were found in these conditions. People with diabetes had a higher risk of heart failure, according to the Framingham studies, regardless of age, body mass index, hypertension, or coronary disease. Higher hemoglobin A1C (HbA1c) in non-diabetic patients increases the risk of heart failure considerably, implicating that pre-diabetic chronic hyperglycemia elevates cardiovascular risk. Obesity also raises cardiovascular disease risk, apart from diabetes, mainly through increased inflammation and reactive oxygen species. Cardiovascular inflammation is usually evidenced by elevated inflammatory cytokines, including TNFα, IL-1β, and IL-6, secreted by immune cells, and reduced anti-inflammatory cytokines, such as TGFβ and IL-10. Cardiac cells, including cardiomyocytes, cardiac fibroblasts, and endothelial cells, also secrete inflammatory cytokines in response to ischemia or hypertrophic stress. Although cardiovascular diseases trigger inflammation at cellular and systemic levels, the heart possesses inherent anti-inflammatory protection. Increasing cardiac function and diminishing inflammation following hypertrophy or ischemia stress can be done through lowering pro-inflammatory mediators or raising anti-inflammatory mediators.

The work of Gussekloo et al points up significant issues, namely that an ideal biomarker such as C-reactive protein (CRP) would ideally be a sensitive risk factor for a specific end-point, say stroke, not for a diffuse group of non-related end-points in order to maximize its positive predictive value. Future research may reveal that CRP belongs to the significant group of risk variables that are linked to a variety of outcomes, including radiation exposure and cigarette smoking. But generally speaking, an association's specificity is a crucial element that frequently indicates a causal relationship. Elevated CRP did not significantly correlate with the death risk from stroke or other cardiovascular illnesses, according to these researchers. The correlation between high CRP levels and risk of several diseases, such as cancer, cardiovascular disease, and infections, is not unexpected considering the relationship between inflammation and CRP. The research revealed a time-dependent association between CRP levels and mortality, where higher CRP levels correlated with decreasing intervals between measurement and death. This finding is consistent with the Cardiovascular Health Study in elderly populations, which differs from research in middle-aged populations. Increased CRP in the elderly could be an expression of acute pathophysiologic processes culminating in clinical disease, disability, and mortality. Subclinical conditions are frequent among the elderly, and thus increased CRP can be an indicator of developing illness, albeit not necessarily an expression of the disease etiology. Although increased CRP can be linked to certain pathophysiologic mechanisms, including thrombosis and clotting, in cardiovascular disease, this study's results indicate that an elevated CRP is linked to a wide variety of outcomes, as opposed to one particular disease process. The correlation between CRP and outcomes suggests that increased CRP can be a marker for nonspecific underlying inflammation and risk of disease, as opposed to a distinct marker for a distinct disease process.

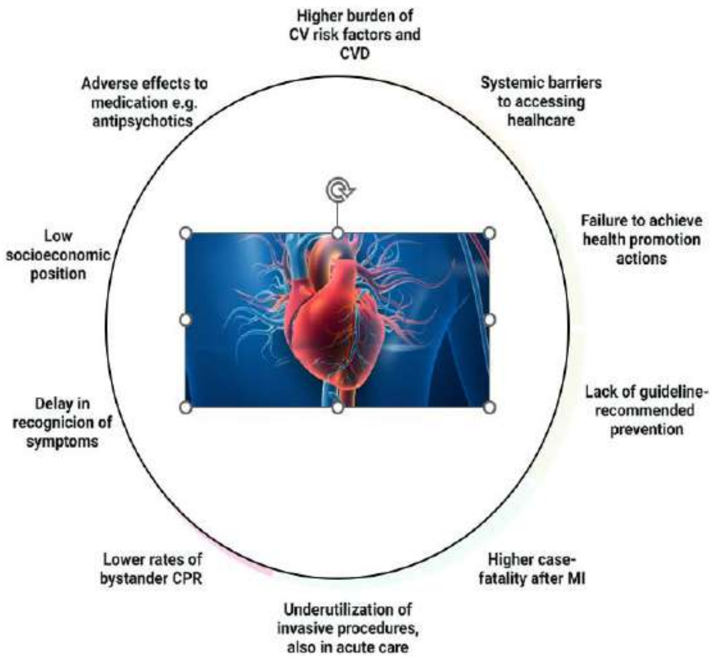

Figure 6: Current Successes and Challenges

CRP may indicate a developing subclinical illness in younger and middle-aged people that is not picked up by standard clinical assessment methods. This subclinical condition could develop into a clinical ailment years later. CRP can be a marker of the severity and progression of unmeasured subclinical disease, but not necessarily a direct causative agent. Elevated CRP is, however, linked with augmented production of proinflammatory cytokines, like interleukin-6, which can directly or indirectly affect disease pathophysiology through their associations with other inflammatory, thrombotic, or fibrinolytic elements.

It would be absurd to assume that CRP and related acute-phase proteins are mechanistic risk factors for disease and that lowering the CRP level will lower the risk of disease without the appropriate data to support such a claim. In spite of the importance of the study of CRP and other acute-phase proteins, as yet there is no firm evidence that CRP is a unique mechanistic risk factor for cardiovascular disease. Moreover, CRP does not seem to indicate the severity or the degree of disease, nor is there evidence to suggest that it is causative. In addition, there is no evidence that lowering CRP or other acute-phase reactants will retard the progression of disease or prevent recurrent disease events. This assertion has been reviewed and approved by the CDC and the American Heart Association's Science

Advisory and Coordinating Committee, on the basis of the key findings and conclusions of the Workshop. The argument is supported by the strongest evidence to date associating cardiovascular disease and inflammatory markers. The study utilized the US Surgeon General's standards for causality, such as consistency, biological plausibility, dose-response relationship, timeliness, and strength. The American College of Cardiology/American Heart Association classification scheme was employed to evaluate strength of evidence supporting the association. In addition, advice on the use of inflammatory markers for screening was assessed employing an evidence-based method, taking into account issues including randomized trial evidence, benefits and harms, performance in various populations, screening methods, and cost-effectiveness.

6. CONCLUSIONS

Atherosclerosis and its clinical consequences are thought to be caused by systemic inflammatory and immunological pathogenesis, according to recent epidemiological, clinical, and imaging research. There is no clinical significance in using atherosclerotic plaque vulnerability traits to predict significant cardiovascular events. A thorough evaluation of atherosclerosis should emphasize a qualitative assessment of a patient's susceptibility rather than anatomical imaging studies of atherosclerotic lesions. Present inflammatory markers are not very specific, and they do not reliably represent underlying biological processes. But longitudinal follow-up of circulating, cellular, and imaging markers could potentially allow identification of high-risk individuals and direct individualized treatment, potentially altering CVD risk. A viable treatment strategy might be to modulate the early stages of atherosclerosis formation and target inflammation upstream. A deeper comprehension of the inflammatory pathways involved in atherosclerosis could result in the development of more targeted therapies without sacrificing the immune system's ability to fight against infections.

Even with effective lipid-reducing medications, residual inflammatory risk persists because methods that target lowering apoB/LDL-C alone do not adequately address the varying inflammatory and/or atherogenic potential across diverse apoB profiles. This emphasizes the necessity of addressing all factors that contribute to atherosclerosis risk, including inflammation, as opposed to evaluating and treating a single marker like apoB/LDL-C. Pharmacological research is being done on a number of medications, specifically those that target the NLRP3-IL1-β/IL-6 pathway. High-risk post-MI patients, who are best identified by an elevated inflammatory load, may benefit most from these medications. However, there is still uncertainty regarding the commencement date and duration of treatment. The prevention of cardiovascular disease could be completely transformed by an all-encompassing approach to residual risk management. However, we are only at the start of this adventure, and there are still many unanswered questions.

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Chapter-IV

IMMUNOLOGICAL RESPONSES TO VIRAL INFECTIONS

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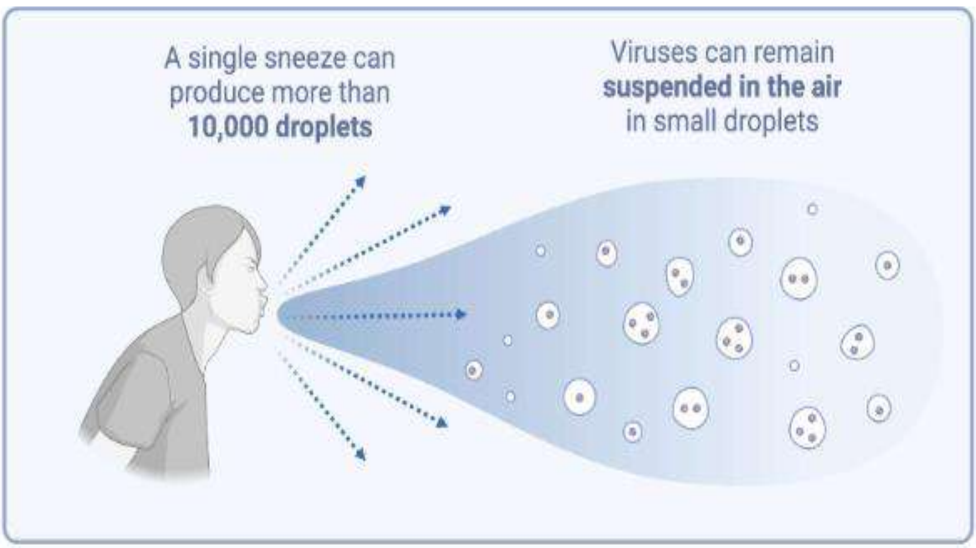
Abstract--- Viral infections are illnesses brought on by microscopic creatures known as viruses that take advantage of your cells in order to multiply. Viruses can infect most other regions of the body, but they are often responsible for respiratory and digestive diseases. Viruses are one type of germ (pathogen) that are so little that they are only visible under a microscope. Each virus carries a small amount of genetic material (DNA or RNA) inside its protective capsid. It can be compared to an envelope with instructions. Conversely, our cells function similarly to a whole factory: They provide all the necessary tools and instructions to complete them. These guidelines advise us how to produce more cells and proteins. Since viruses lack cells, they lack all the "machinery" necessary to replicate themselves, unlike you and me. Therefore, in order to repeat their instructions, they must enter our cells and utilize our machineries. What causes a viral infection is the replication of viruses. Adaptive immunity arises after being exposed to an antigen from an illness or vaccination. Compared to the adaptive, or acquired, immune response, which takes days or even weeks to develop, the innate immune response happens far more quickly.

Keywords--- Viral Infections, Immunity, Immune Response, Influenza Virus.

1. INTRODUCTION

We are exposed to several viruses that might infect and cause disease in the course of our daily lives. Usually, viral invasion occurs on the mucosal surfaces lining the gastrointestinal, respiratory, and genitourinary tracts. Without adaptive immunity to viruses, a primary infection can develop into a disease. Depending on the type of system triggered, the immune system's reaction to a virus is typically classified as either an innate or adaptive immune response. The innate immune response provides a more comprehensive representation of the host's response to many pathogenic diseases. Limiting the spread of illness and preventing viral replication are the goals of the innate immune system. The innate immune response employs specific strategies based on pattern recognition receptors (PRRs) to recognize viruses or viral particles. The same kinds of microbes share conserved microbial structure, which these receptors recognize. Certain metabolic products, which are traits of a certain microbial type, are essential components of PRRs. When it comes to viruses, the infected cell produces the different viral compounds. However, viral nucleic acids are the primary targets that PRRs recognize. The viral RNA is also detected by the innate immune system (Hottz et al., 2018). The host cell's cytoplasm and endo-lysosome contain viral nucleic acid. However, host DNA is absent from these locations. Both virus DNA and RNA sensing systems are found in the cytoplasm. The cytoplasmic immune response may be triggered by this sensing mechanism. Changes in cellular processes, such as an abrupt lack of host protein synthesis, ER stress, or altered ion channel activity, can also function as sensing mechanisms (Chen et al., 2018). The innate immune system uses a two-step eradication process after identifying the infection. Initially, it triggers an antiviral reaction, which is mostly mediated by type 1 interferon (IFNs). Second, it may trigger the adaptive immune response, which would lead to both antigen-specific and long-lasting antiviral immunity. The primary goal of the host's reaction to a viral infection is to eradicate the infected cells.



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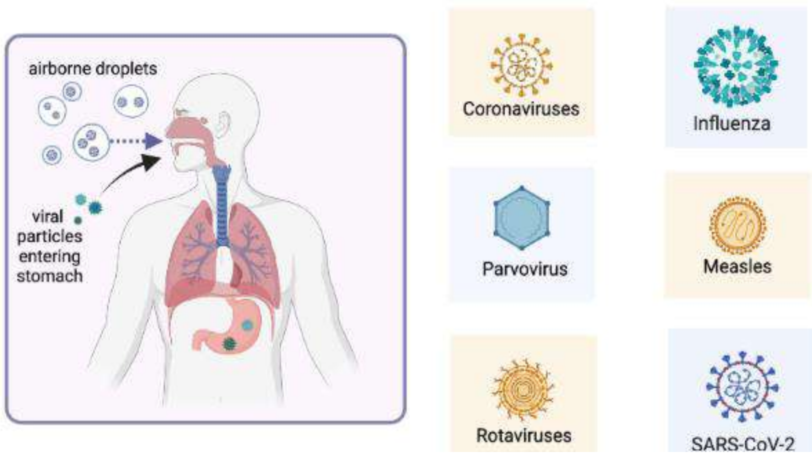

(b)

Figure 1: Viral Infections (Source: Web)

As obligatory intracellular parasites, viruses need their host to reproduce and distribute them to other people. Even while viral infections are extremely cytolytic to individual cells, they are rarely fatal in people. When virus's cross species (like HIV or Ebola), decrease host immunity, or drastically alter their antigenic composition (like influenza viruses), they frequently result in mortality. HIV is among the most notable examples in human history of an alien virus that kills its host. However, HIV is a valuable strategy for species persistence since it dies slowly, giving it lots of opportunity to infect new hosts. It is evident that in mammals with insufficient immunity, the death or serious consequences that accompany a viral infection occur when fetuses or neonates, especially those without passive immunity, succumb to different substances that are well tolerated by healthy adults (Pecora et al., 2020). Sensitive techniques for determining the significance of immune pathways for antiviral defence have been made possible by the growing abundance of immunological tools, such as major histocompatibility complex (MHC) tetramers and transgenic animal models. Usually, several immune components work together to protect against viruses, and the efficiency of a single mechanism varies greatly based on how various viruses enter, proliferate, and spread within the host.

2. MONITORING OF VIRAL INFECTIONS

There are several methods for identifying viruses in animals. One of the widely used techniques for identifying the pattern linked to viral nucleic acid is PRRs. Depending on the type of cell involved—infected or not—the innate immune approach of viral identification includes both cell intrinsic and cell extrinsic components. The cell sensing system includes RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs). These receptors are usually activated in infected cells. Transmembrane receptors called TLRs (Toll-like receptors) and CLRs are engaged in cell extrinsic recognitions that are mediated by plasmacytoid dendritic cells, dendritic cells, and macrophages.

Cell Self-Recognition of Virus

Melanoma differentiation-associated gene 5 (MDA 5), retinoic acid-inducible gene 1 (RIG 1), and cytoplasmic RNA helicases are markers of RNA virus infection in cells. Numerous cell types have RIG 1 like receptors, which are able to identify viral RNA helicase domains. Additionally, RIG 1 has a catalytic domain to start antiviral signaling and a repressor domain at its Cterminus that inhibits it. The membrane protein known as mitochondrial antiviral signaling (MAVS) protein serves as an adapter for the peroxisome and mitochondrial membrane-dwelling MDA5 and RIG 1 proteins. Two protein kinases that MAVS targets are caspases 8/10 and (FADD).

Cytoplasmic Viral DNA Sensors

For DNA from different microorganisms, such as bacteria, viruses, apoptotic host cells, and synthesized B form DNAs, such as poly dA, there are several DNA detecting mechanisms. Through NK-kB and IRF3, the dT B form of DNA activates type 1 IFN. DAI, often referred to as DLM 1 /ZBR1, is a molecule involved in intracellular DNA sensing.

Cyclophilin A Signature of Viral Infection

Viral proteins can occasionally be used as indicators of viral infection. A peptidyl prolyl isomerase called cyclophilin A catalyses the cis/trans isomerization of target proteins' x proline epitopes. For dendritic cells to respond innately to HIV 1, they must come into contact with newly produced HIV 1 capsids and have cyclophilin A present, which causes a type 1 IFN response. Nevertheless, CD4+ cells lack this dendritic cell capacity. Because human DC are resistant to HIV-1 infection, this pathway is not used in humans to produce type 1 IFN. Because viruses without this would be unable to reverse-transcribe viral RNA, cyclophilin A is integrated into the HIV 1 virion by attaching to the capsid protein of HIV-1.

Role of TLR

Toll-like receptors (TLR) are utilized by most bacteria as pattern recognition receptors against chemical patterns that have been conserved throughout evolution. The number of TLRs in a cell varies; in humans, there are 10 functional TLRs, but in mice, there are eleven (Murtaugh et al., 2002). In essence, all TLRs are type 1 transmembrane proteins that use an extracellular domain with a leucine rich repeat (LRR) to identify pathogen-associated molecular patterns (PAMPs). The transmembrane domain of a TLR determines its position, whereas the cytosolic domain contains the toll interleukin 1 receptor domain (TIR). This domain produces pro-inflammatory and antiviral cytokines and activates the adaptor protein. Genes that inhibit viral replication were activated as a result of type 1 IFN production.

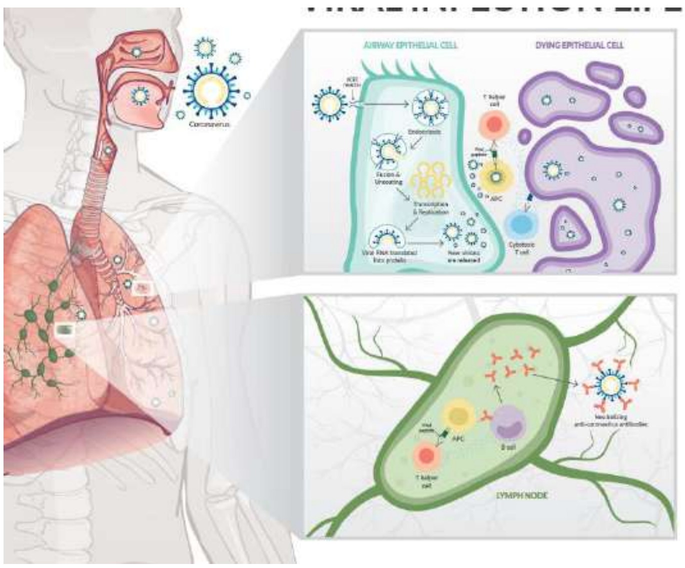

Figure 2: Viral Infection Life Cycle

Role of C Type Lectins

In addition to TLR, the plasma membrane contains another kind of receptor that can attach to viral antigen via its carbohydrate recognition domain and start the binding of Ca2+-dependent ligands. C type lecitin (CLR) receptors are these receptors. Different types of CLR have different functions. For instance, some CLRs do not bind to Ca2+, while others are utilized to induce signals. Other CLRs prefer to cause pathogens to be endocytosed. Mannose receptor DEC 205 and langerin are two CLRs involved in endocytosis; both are expressed in CD8+ DC and Langerhans cells, respectively. By attaching itself to Langerin molecules found on Langerhans cells, HIV viruses are eliminated. Dectin 1 and DC SIGN are components of Signal Inducing CLR. DC SIGN binds to HIV1 and the Ebola virus.

Role of Tetherin and Viperin

Tetherin is highly expressed when IFN (type I) is present. In the absence of viral protein unique (VPU), it prevents the release of retrovirus particles. Tethrin inhibits the release or retrovirus particle by binding the lipid and coordinating the change. The betaTrCPE3 ubiquitin lipase complex is used by VPU to trigger endosome trafficking, which eliminates the tetherin from the cell surface and prevents it from releasing the enveloped virus. Tetherin restricts the spread of filovirus and retrovirus (Braciale et al., 2012). Endoplasmic reticulum associated interferone inducible, or viperin, is a virus inhibitory protein that is produced in response to IFN and is in charge of preventing the spread of viruses such as hepatitis, dengue, HIV, influenza, and alpha viruses. Viperin inhibits farnesyl diphosphate synthase activity, which disrupts influenza virus release by disorganizing lipid rafts.

Role of APOBEC Family in Innate Immunity APOBEC

A kind of cytidine deaminase with one to two catalytic deminase domains is called Apalipoprotein BmRNA-editing catalytic polypeptide (APOBEC). When type 1 and type 2 IFN are stimulated, APOBEC expression rises and is constitutively expressed in a variety of cell types.

Role of RNA Interference

In certain plants, fungi, insects, and nematodes, RNA interference (RNAi) is a key antiviral defensive mechanism. A molecule called the dicer endonuclease family identifies viral dsRNA by cleaving it into siRNA in the initial step of RNA interference. The RNA-induced silencing complex (RISC) incorporates siRNA in the second stage. In order to degrade and cleave viral RNA, this complex directs the RNAase enzyme AGO (Agronaute protein) to a complementary sequence in the virus. RNA interference is not present in virus-infected mammalian cells. The absence of RdRPs and siRNA amplification in mammalian cells indicates a lack of a systemic antiviral

RNAi response. Nonetheless, the antiviral response involves a number of different cell types. NK cells are in charge of identifying and getting rid of virus-infected cells. They also release IFN-γ, which starts other antiviral mechanisms. Activating and inhibitory receptors are the two different kinds of receptors found on the cell surface of NK cells. They either start or stop their target cell from lysing. The chemicals that block NK cells via inhibitory receptors are derived from class 1 M.H.C., which is downregulated during viral infection.

3. ADAPTIVE IMMUNITY TO VIRAL INFECTION

In order to infect new cells within the host body, the virus uses its biosynthetic machinery to multiply within the host cell. During an acute infection, the virus's capacity to multiply and the host cell's ability to survive compete. that the virus has two possible outcomes: it can either spread and kill the host or it can linger in the body for weeks, months, or even years (Russell et al., 2017). This entire event led to immune suppression and virus persistence in the host humans, which was an ecological burden for the viruses. Two viruses that have co-evolved with humans are Epstein Barr Virus (EBV) and Herpes Simplex Virus (HSV). They have developed latency to suppress the host response and have persisted in the human population. Numerous other viruses have developed ways to keep their levels low in afflicted people and spread by infecting those who have never been exposed before. These people become carriers of infections and acquire mild or serious diseases. Certain animal viruses have the ability to spread from their native host to humans, and they are the cause of significant pandemics that result in zoonotic illnesses in people. The HINI influenza virus, for example The SARS coronavirus causes severe acute respiratory syndrome, HIV causes AIDS, and a virus causes swine flu. Although the majority of viral infections damage tissues or organs, some viruses, including the human papilloma virus, hepatitis B, hepatitis C, and human herpes virus 8, can cause cancer.

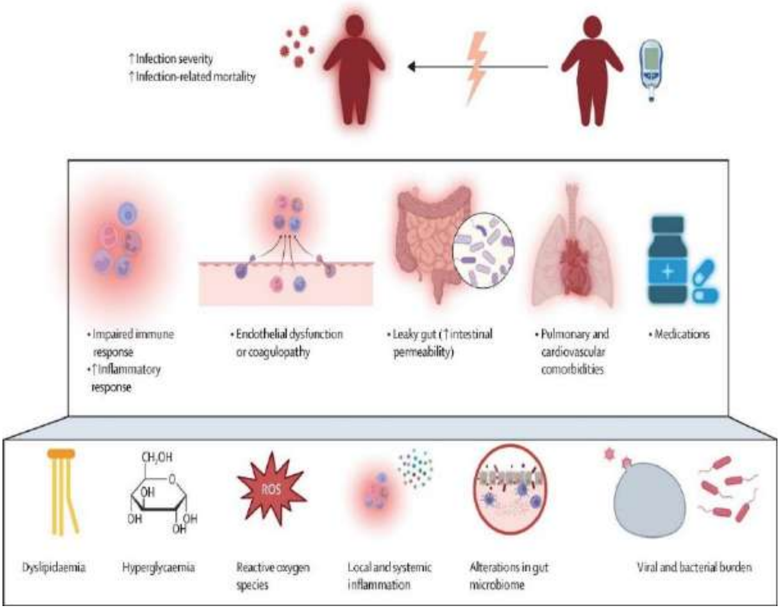

Figure 3: Mechanism of Viral Infection

In Sight to Adaptive Immunity to Virus Infection

The adaptive system uses B and T cells to identify the viral components and requires a few days to learn about the type of virus infection (Welsh et al., 2004). Through their B cell and T cell receptors, which are expressed by a small percentage of cells against a certain virus, they attach to the viral components. They are therefore in charge of creating immunological memory.

B Cell Response to Viral Antigens

Both soluble and membrane-bound viral antigens can be recognized by B cells. The majority of antigens in viral infections are actually presented by antigen presenting cells (APCs) following antigen processing and presentation. B cell activation or proliferation is the outcome of B cell binding to viral antigen. Additionally, it causes the viral antigen to be internalized by the B cell receptor and cleaved by the endocytic pathway. The B cell surface will display the antigenic peptide associated to the M.H.C II molecule, which will be recognized by viral-specific T cell receptors (TCR) on CD4+ cells (T helper cells), also referred to as T follicullar helper cells or TFH. This may result in B cell proliferation and T helper cell cytokine release. T cell engagement is not necessary for B cell activation to occur. T-independent antigens are the name given to these viral antigens. Only when 1gM molecules cluster and attach to antigen can B cell receptors (BCR) result in signal transmission. The cytoplasmic regions of molecules 1gα and 1gβ, which are connected to two additional membranes, include an immune-receptor tyrosine activation motif. Tyrosine is phosphorylated as a result of BCR clustering, and B cells then use their antibodies to attach to the virion (Wodarz et al., 2002). The M.H.C II molecule displays the cleaved peptides on the B cell surface once the entire virion is consumed. The B cell attaches itself to the T cell once the T helper cell is presented with this antigenic peptide. B cells' CD 40 receptor and TFH cells' CD 40 ligand are both impacted by CD 40 and its ligand. Cytokines and viral co-stimulatory receptors from this cell-to-cell interaction promote B cell growth.

Recognition of Viral Antigen by T Cells

Following the presentation of this antigenic peptide to the T helper cell, the B cell adheres to the T cell. CD 40 and CD 40 ligand interact with the receptor CD 40 found on B cells and the CD 40 ligand on TFH cells. Virial co-stimulatory receptors and cytokines from this cell-to-cell interaction aid in the proliferation of B cells. The M.H.C.I. molecule presents these viral proteins to CD8+ cells after they have first been ubiquitinized and broken down by a proteasomal complex via a cytosolic pathway. The viral antigens on the viral surface are bound by humoral antibodies, which neutralize them. As an alternative, they identify the antigen on the viral surface and stop the virus from attaching itself to the host cells.

Viral Antigen and its Neutralization by Antibodies

Plasma secretes immunoglobulin (Ig), which stops viruses from attaching to host cells and stops infections from happening again. IgG, IgA, and IgM antibodies stop the viral envelope from fusing with the host cell. Certain antibody classes encourage the phagocytosis of viral particles. Complement activation and opsonization by C3b, followed by viral envelope lysis, can be brought on by IgG and IgM.

B Cells and Primary Viral Infection

In the absence of T helper cells, B cells alone are responsible for controlling certain viral infections brought on by the polyoma virus, vesicular stomatitis irus, and rotavirus. This is only made possible by the viral antigen being repeated on their surface, which allows IgM antibodies to neutralize them when T helper cells are not present. When the entire virion is consumed, the cleaved peptides are visible on the surface of the B cell as the M.H.C II molecule. After the T helper cell is exposed to this antigenic peptide, the B cell binds itself to the T cell. CD 40 and its ligand affect both the CD 40 receptor on B cells and the CD 40 ligand on TFH cells. B cell proliferation is aided by cytokines and viral co-stimulatory receptors from this cell-to-cell interaction (Braciale & Hahn, 2013). A Fla virus infection demonstrates that antibodies alone are adequate for managing the infection; but, if an infection has persisted, a T cell response is frequently thought to be beneficial for viral infection eradication.

B Cell and Secondary Viral Infection

A premade antibody is more effective at protecting against a variety of viruses. Blood-borne antibodies from the mother shield the fetus from respiratory syncytial virus, herpes virus, influenza virus, rubella polio virus, and hepatitis B. These antibodies are quite powerful and can shield the newborn from viral infections that, in the event of primary infections, can only be managed by CTL.

4. CHANGE IN ANTIGEN DISPLAYED ON SURFACE

Certain viruses constantly alter the antigen they present in order to avoid immune system identification. For instance, influenza viruses evolve new strains by altering their surface antigen. Recurrent infections happen as a result of strain type changes (Guidotti & Chisari, 2001). Because HIV mutates more quickly than influenza viruses, its antigen exhibits a greater degree of diversity.

Ability of Complement Inhibition

When viruses attach themselves to antibodies, an antigen-antibody complex is formed. The lysis of the viruses, which are subsequently eliminated by phagocytosis, is only possible when complement proteins are present. Viruses have developed defences against these annihilations caused by complement. The complement control protein known as Vaccinia Complement Control Protein (VCP), which is generated by vaccination viruses, promotes factor I to cleave C3b and C4b, blocking the traditional complement activation route.

Cytokine Inhibiting Proteins

Many viruses have the ability to produce proteins that circumvent both host-specific and non-specific defense mechanisms. Many cells that are infected by viruses produce cytokines like IFN α and β. In neighboring cells, IFN α and β are in charge of producing antiviral proteins. Virus associated RNAs I (VAI) is the name of this antiviral protein. By blocking the activity of DNA dependent activator of IRFs (DAI), adenovirus and Epstein-Baa virus circumvent the antiviral effect of VAI.

Degradation of Proteins

After being broken down by proteolysis, the antigenic protein is visible on the cell surface of Epstein-Barr virus (EBV)-infected cells. Glycine-alanine repeats seen in the viral protein EBNA I, which is encoded by EBV, disrupt the proteasomal complex. The human cytomegalovirus (HCMV) causes infected cells to create a viral protein called phosphoprotein 65 (pp65), this stops T cell receptors from producing antigenic peptides.

MHC Class I Down Regulation

Class I MHC plays a vital role in the removal of virus-infected cells by presenting antigenic peptide to CD8+ cells, also referred to as cytotoxic T-lymphocytes (CTLs). Aswathy et al., (2015) Certain viruses reduce the expression of class I MHC, which prevents it from showing up on the cell surface. Because cytosolic pathway proteins like TAP and LMPs are downregulated, the virus-infected cell is able to evade CTL activity.

MHC Class I Molecule Retention in Cell

MHC class I may be retained in the endoplasmic reticulum (ER) or transferred out of it. Once MHC class I reaches the cell surface, viral proteins can change its structure.

5. KEY IMMUNE CELLS AND CYTOKINES INVOLVED

In order to generate an initial infection that can lead to illness, viruses infiltrate human cells. The body is protected from these diseases by the immune system. To fight the invasive virus, human cells require both innate and adaptive immune responses. When viruses, viral particles, or both are detected, the immune response starts. There are several ways for mammals to do this. Both intrinsic and extrinsic mechanisms are possible. Pattern recognition receptors (PRRs) are used in both situations. The conserved microbial structure present in the same groups of bacteria is recognized by PRRs, which are receptors.

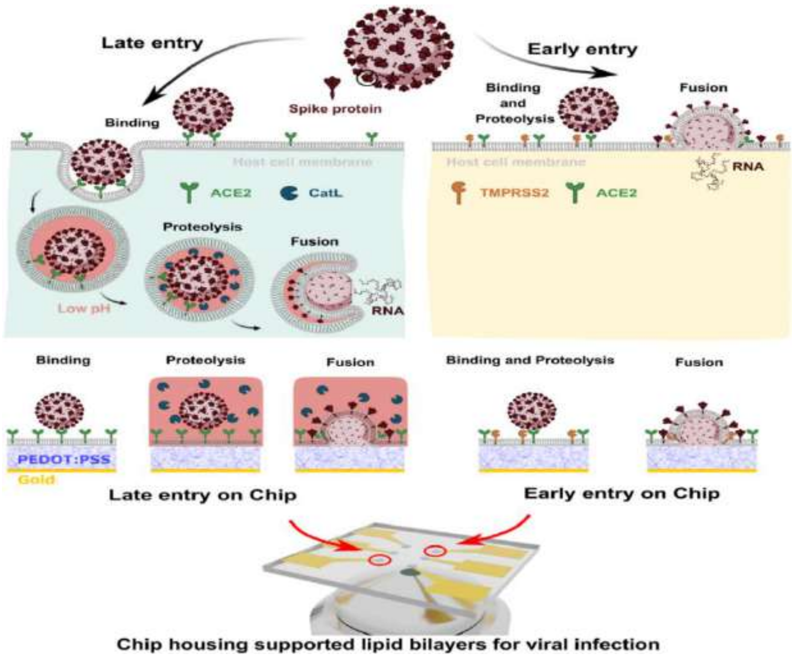

Figure 4: Biological Cycle of Viral Infection

As a result, cells keep an eye out for viral particles (DNA, RNA, and viral proteins). The two crucial PRR classes identify the viral particles and initiate the downstream Type-C lecithin receptors (CLRs) and Toll-like receptors (TLRs) are immune responses. When a virus or viral particles are recognized, the adaptive immune response is activated and an antiviral response mediated by type-1 interferons (IFNs) is induced. Pro-inflammatory and antiviral cytokines are produced when TLRs are activated, and endocytosis of viruses and viral particles occurs when NFkB is activated (Zhang et al., 2020). Additionally, several molecules that play crucial functions in immunization against viral infection are activated. These molecules include theterin, which is an endoplasmic reticulum interferone associated protein that disrupts the virus's lipid raft, and viperin, which is a lipid raft linked protein that restricts the release of viral particles. RNA interference, or iRNA, is also essential for identifying viral dsRNA and using dicer endonucleases to cleave it into small interference RNA (siRNA). The RNAase enzyme Argonuate protein (AGO) breaks down these siRNAs once they are integrated into RNA-induced silencing complexes.

Certain types of white blood cells are activated when virus particles attach to their epitopes with antibodies in our bodies. B cells multiply to create memory cells and plasma cells, which generate antibodies and contribute to long-term immunity. Two methods are used, depending on how well the viral genome integrates into the infected cell. When the viral genome and the subsequent endocytotic route are not integrated, the cytosolic pathway occurs. To prevent the infection from spreading, the viral genome is digested in both cases to create MHC II and MHC I molecules, respectively, which identify the cell for destruction or death.

6. Immunological Mechanisms of Viral Evasion

Viruses manage to elude the immune system despite the immune system's many defense mechanisms against viral infections. In order to accomplish this, they either alter the antigen that is visible on the cell surface, interfere with proteosomal complexes, block complement pathways, inhibit cytokine activating proteins, or downregulate MHC I molecules.

The majority of human viruses face several barriers to entry and infection when they reach their target sites. The most efficient of these are the mechanical barriers provided by the skin and mucosal surfaces and the chemically hostile environment of the gut. Numerous common human viral infections, including rotavirus, enteric adenoviruses, and hepatitis A virus (HAV), enter the body through the gastrointestinal tract. Usually, tainted food and beverages or social contact are how they are spread. Influenza viruses, rhinoviruses, coronaviruses, measles viruses, varicella-zoster virus (VZV), and respiratory syncytial virus (RSV) frequently spread respiratory infections by aerosol transmission and person-to-person contact. The skin or mucosae are the target of several herpes viruses, such as VZV and herpes simplex virus (HSV). HSV in particular can infect the skin, the eye, and the mucosa of the mouth and genitalia through microscopic wounds and abrasions (Orefice, 2021). A number of herpes viruses, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV), attack the mucosa. In rare instances, CMV can also spread vertically from mother to kid or through blood transfusions. The human papillomavirus (HPV) can cause warts and alter cells on the skin and muscosa, which can result in cancers like cervical cancer. Viruses such as the Semliki Forest virus and the West Nile virus can also enter via the skin thanks to insect vectors. One common method that HIV and the hepatitis B virus (HBV) spread is through sexual interaction. HIV, HBV, and the hepatitis C virus (HCV) may enter the bloodstream directly through contaminated needles or transfusions.

In order to survive and proliferate, a virus must enter its host's cells. The cells of the immune system cannot "see" the virus once it has entered, thus they are not aware that the host cell is infected. To get around this, cells use molecules called class I major histocompatibility complex proteins, or MHC class I for short, to display protein fragments from inside the cell on the cell surface. Cells can communicate with one another regarding their contents thanks to this technique. If the cell is infected, these peptide fragments will contain parts of viruses' proteins. Because viruses are so adaptive, they have developed ways to avoid being recognized by T cells. Some viruses block the presentation of viral peptides on the cell surface by MHC molecules. In this instance, the T cell is not aware that the virus is present in the infected cell. An immune cell that focuses on destroying cells with fewer MHC class I molecules on its surface is known as a natural killer cell, or NK cell for short. When NK cells identify a cell displaying less than normal MHC molecules, they release lethal chemicals, much like cytotoxic T cells do when they eradicate the virally-infected cell.

Preformed mediators are a weapon for cytotoxic cells. Both cytotoxic T cells and NK cells include granules, which are compartments that hold cytotoxic components until they come into contact with an infected cell and are released. One of these mediators is the protein perforin, which can create holes in cell membranes that let other substances enter a target cell and aid in the cell's demise. Additionally, the granules store and release enzymes known as granzymes. Granzymes enter the target cells through the holes made by the perforin.

Virally infected cells create and release small proteins called interferons, which help the immune system fight against viruses. Interferons prevent viruses from multiplying by directly blocking their ability to grow inside an infected cell (Kim et al., 2011). They also act as signaling molecules, allowing infected cells to notify nearby cells of the virus's existence. As previously discussed, this signal prompts nearby cells to create more MHC class I molecules on their surfaces, which helps T cells identify and eliminate the viral infection.

Consequently, different parts of the body may contain viral antigens, depending on the stage and mechanism of transmission of the infection.

These cells are not killed by the infectious process, even if they produce a lot of viral offspring and have virus-specific antigens on their surface. In some steady-state infections, the virus can spread from cell to cell without coming into touch with the outside world. The virus breaks through the cell membrane to unleash its offspring. While DNA viruses cannot establish steady-state infections, some RNA viruses, such as retroviruses and parmyxoviruses, can.

On the surface of infected cells, virus-specific antigens may be expressed. The virus in these cells has the ability to multiply, reactivate, and cause illness months to years after infection. We still don't fully understand the mechanisms that allow viruses to remain intracellular for extended periods of time before reactivating. The immune system is thought to have a hard time identifying sick cells in sequestered parts of the body, such the brain system, where many latent infections develop. Furthermore, the immune system does not detect any cell that has a virus but does not express viral antigens.

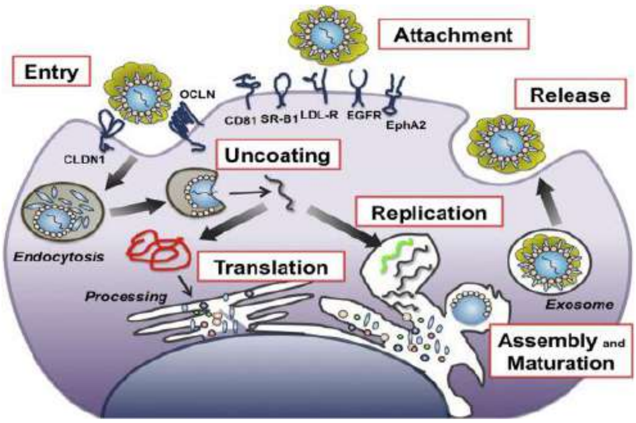

Figure 5: Immunological Mechanisms of Viral Evasion

Integrated Virus Infection

When all or part of the viral nucleic acid is integrated into the host cell's genome, it is known as an integrated virus infection, which is a distinct type of persistent virus-host cell interaction. On the other hand, the cell surface or inside the cell may include novel virus-specific antigens. One well-known example of this process is retrovirus infection.

Virus Spread

The manner a certain virus travels within the host is another crucial factor in how viral infections cause an immune response. There are four known forms of viral transmission in animal hosts:

(1) Local spread, where the infection is mostly contained inside a mucosal surface or organ (e.g., rhinoviruses infecting the respiratory epithelium or rotaviruses infecting the gastrointestinal epithelium);
(2) Primary hematogenous dissemination, where the virus spreads to target organs after being directly injected into the bloodstream (e.g., viruses spread by insects);
(3) secondary hematogenous transmission, in which a virus replicates and infects a mucosal surface initially, usually without causing any symptoms, and then spreads to target organs through the bloodstream (e.g., poliomyelitis, mumps, and frequent viral exanthems); and

Similarly, local mucosal immunity is not always the outcome of systemic immunity. For instance, serum IgG is the main antibody produced by the systemically injected Salk polio vaccine, which uses a dead virus and causes little to no secretory response. Because there is no secretory antibody, the virus may stay at the intestinal portal of entry, causing the immunized person to become a transient carrier but preventing systemic infection. On the other hand, the live, oral Sabin polio vaccine produces secretory antibodies in the intestine and effectively stops the virus's multiplication and subsequent mucosal penetration.

Multiplicity of Immune Defences

The host immune system's defences against viral infections are extremely sophisticated, according to recent research. The host immune defences' numerous components and their interrelationships give rise to this complexity. Given the diversity of viruses, hosts, infection pathways, body compartments, cells, and virus replication and dissemination processes, it is not surprising that different defences exist (Mohamed et al., 2019). The problem is further complicated by the varied efficacy of the various host defences during resistance to reinfection and the many stages of the first viral infection. Moreover, disease symptoms may be brought on by the host's defences being triggered. The fact that each virus has many defences helps explain why a host's resistance to viral infections is not completely eliminated when one or a few defences are compromised. A number of host defences, both immunological and nonimmune, may work to prevent viral infections or, occasionally, exacerbate the illness. Although a lot of the immunological defences against viral invasion are fairly well recognized, further research is needed to determine how effective each one is in comparison. In particular, humoral and cell-mediated immunity are not distinct but rather interact closely to influence the intensity and duration of each immune response, as this chapter seeks to make clear.

Following vaccination or viral infection, different antibody classes emerge one after the other. For instance, most antigens initially cause IgM (early antibody) responses after primary infection or immunization; IgA and IgG responses come a few days later. On the other hand, reinfection primarily promotes the synthesis of IgG, albeit some IgM and IgA are also produced. IgA antibody predominates, with some IgM present, when the respiratory or gastrointestinal system is the site of the principal antigenic stimulation.

It has been demonstrated in immunocompromised animals that the length of viremia is associated with the likelihood of central nervous system invasion. In the majority of these hypogammaglobulinemia patients, the majority of viral infections have a benign course, suggesting that their poor antibody response and other distinguishing factors may work well.

7. CONCLUSION

This paper discusses the importance of noncytolytic processes for managing viral infections, paying particular attention to the role of cytokines. This can limit the virus's amplification and spread and attenuate the infection. However, other immune response mechanisms are necessary to eradicate established viral infections. However, adaptive immunity targets invasive pathogens more specifically. A particular arrangement of eight amino acids in a protein that is exclusive to influenza viruses, the virus that causes "the flu," is an example of an antigen. The bacteria that cause meningitis, Neisseria meningitidis, has a short chain of carbohydrates on its cell wall that serves as another illustration. Millions of possible amino acid, carbohydrate, and other small molecule sequences exist that could function as antigens. The ability of the immune cells that make up the adaptive immune system to identify and react to a single antigen or a small number of highly similar ones makes it function. Indeed, they can eliminate viruses from infected cells noncytopathically as long as the virus is vulnerable to these cytokines and the cell has the ability to activate antiviral mechanisms. Importantly, the same cytokines also indirectly control viral infections by altering the activation, amplification, recruitment, and effector actions of the immune response, as well as by upregulating antigen processing and viral epitope display on the surface of infected cells. Considering these theories, it is not surprising that a number of viruses encode proteins that could inhibit the antiviral actions of cytokines.

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Chapter-V

NOVEL THERAPEUTIC STRATEGIES FOR ALZHEIMER'S DISEASE

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Abstract--- Alzheimer's disease (AD) is an advanced stage of dementia that gradually worsens memory loss, impairs thinking abilities, and makes it harder to complete basic tasks. The disease is called Dr. Alois Alzheimer, in the year 1906. The loss of connection between neurons in the brain, which transmits information to the body's organs, is another effect of AD. The hippocampus, which stores memories, is first affected by AD. Later, it spreads to other parts of the brain and causes it to shrink. The brain would have considerably decreased by the time AD reached its most severe stage. It has been noted that not every memory loss issue results in AD. The majority of AD symptoms vary from patient to patient and are not universal. Not all patients with moderate cognitive impairment (MCI) develop Alzheimer's disease (AD), despite MCI being a warning indication of AD. Because AD patients may not be able to perform their own responsibilities, the majority of them get anxious, and some of them become more aggressive. These drugs simply stop the decline of cognitive capacities and momentarily relieve symptoms. In this article, we first address the drawbacks of anti-Aβ treatments, such as their low effectiveness and possible side effects, which emphasize the need to look into different targets and processes. We next go over intriguing non-Aβ-based approaches such gene and stem cell therapy, neuroinflammation management, and tau-targeted therapeutics. These approaches offer new therapeutic possibilities for AD by tackling downstream consequences and other disease markers besides Aβ buildup.

Keywords--- Alzheimer's Disease, Dementia, Neuroinflammation, Amyloid, Tau, and Aducanumab.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia among the elderly, accounting for up to 70% of cases. In 2010, there were about 36 million AD cases worldwide, but the number of new cases is increasing by 7.7 million annually (Wang & Zhang, 2018). By 2050, there are projected to be 144 million AD patients worldwide (Gomez-Ramirez and Wu, 2014). One in three elderly people dies from Alzheimer's disease or a related dementia. According to the Alzheimer's Association (2013), AD is currently the sixth most common cause of death among all illnesses. According to the Alzheimer's and Related Disorders Society of India (2010), 50–75% of the 3.7 million Indians who have dementia have severe AD, and by 2030, this number is predicted to quadruple. The most prevalent type of age-related dementia, AD, is typified by a progressive loss of memory and deteriorating cognitive function, which eventually results in a decline in bodily functions and death. The neuropathological features of AD include senile plaques and protein clusters termed neurofibrillary tangles that are created in the brain. The intracellular bundles of paired helical filaments known as neurofibrillary tangles are mainly composed of tau, a microtubule-associated protein, which is abnormally phosphorylated (Ibrahim & Gabr, 2019).

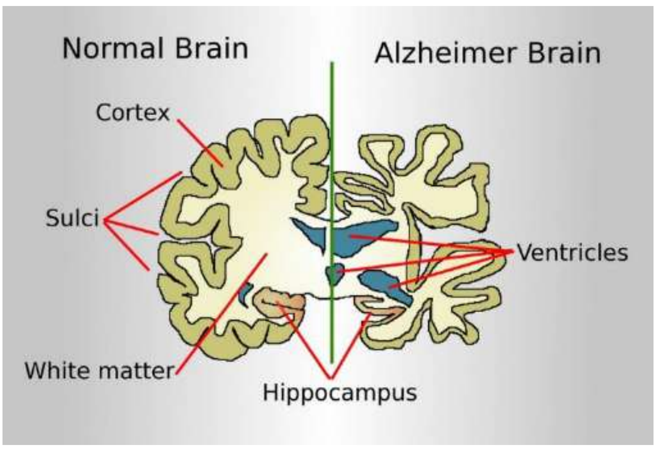

Figure 1: Alzheimer Disease

Dementia is not a huge health concern because of the aging population. Multiple cognitive deficiencies that are severe enough to interfere with day-to-day activities describe this clinical condition. The deficits include impairment of memory and any other cognitive domain disorder like agnosia, apraxia, aphasia, and so on. 60 to 70 percent of all cases of dementia in the elderly are caused by Alzheimer's disease (AD). A combination of several factors has made this a relevant issue in science and public health. The need to identify prophylactics that can reduce risk or delay the onset of this is critical from the standpoint of choices of lifestyle. Here is an increase in a body of evidence to support the premise that intake of coffee/caffeine can bring down the risk of AD or delay its onset. This began with an epidemiologic human study and was supported by a controlled study in AD transgenic mice (Ramesh & Govindaraju, 2022).

These studies gave an insight into the mechanisms where coffee/caffeine protects against AD even in the case of Mild Cognitive Impairment (MCI). The studies of Epidemiology have supported coffee/caffeine as being protective against such impairment and AD these studies though insightful will not be providing direct evidence to the prophylactic effect of coffee/caffeine against AD as this is all largely based on the recall which will not be able to isolate unequivocally the intake of coffee/caffeine from that of the other factors which may affect cognition over an entire lifetime (if they are not fully controlled). Luckily, creating the AD transgenic mice has permitted a highly controlled study that was performed to delve into the details of AD pathogenesis as well as its therapeutic development.

The loss of both the structure and function of neurons is generally referred to as neurodegeneration. Although neuronal degradation is the primary cause of all diseases categorized as neurodegenerative disorders (NDDs), each disease affects a particular subset of neurons either physically or functionally. In addition, each neurodegenerative disease is thought to have unique pathogenic processes or causes of illness that culminate in neuronal death. However, certain diseases exhibit common symptoms due to overlap of the affected regions, either as a result of the disease's course or because of pathological commonality. Too far, over a hundred neurodegenerative diseases have been identified. However, for the above stated reasons, their classification is still difficult. Typically, diseases are categorized based on the pathological hallmarks, clinical presentation, or the area of the brain that is afflicted.

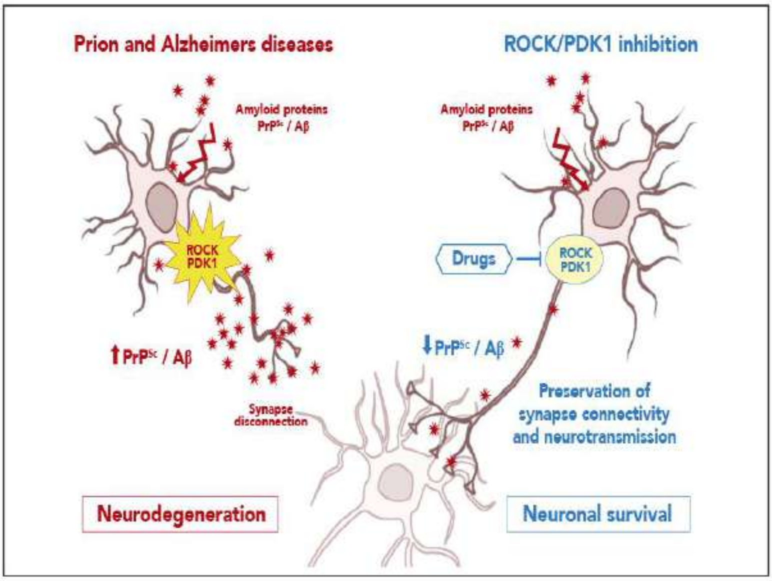

Figure 2: Prion Alzheimer Disease

Age of Onset of AD

While early-onset AD usually appears at age 60 or younger, sporadic AD frequently appears at age 65. In order to evaluate changes in amyloid neta biomarkers, APOE4 carriers with a parental history of sporadic AD may find it helpful to be close to parental symptoms, per a recent study (Villeneuve S. et al, 2018).

Behavioural Symptoms of AD

Memory loss is the most typical sign of AD. New information, significant dates and occasions, individuals, languages, and the use of specific drugs can all be forgotten by patients. Disorientation in time and space is the second most prevalent symptom. Patients occasionally struggle to carry out duties in a familiar location on their own and frequently forget frequently used routes and locations. Planning and problem-solving are often difficult for AD patients. There have been a few documented instances of AD patients with visual impairments, such as trouble reading, determining an object's distance from them, distinguishing colors, and comprehending contrast.

Each AD patient has psychotic symptoms at a different frequency and with varying degrees of aggression or intensity. According to Rosenberg et al. (2015), agitation, apathy, depression, and psychosis (delusions) are the most prevalent neuropsychotic symptoms of AD. Twenty percent of all MCI patients in one population-based study experienced depression, twenty percent displayed significant apathetic symptoms, and fifteen percent displayed extreme agitation. 30 percent of dementia patients displayed indicators of agitation and violence, 32 percent of patients experienced depression, and 36 percent of patients displayed indifference.

2. PATHOLOGY OF ALZHEIMER'S DISEASE

The brain can compensate for neural dysfunctions because of a certain amount of redundancy. The symptoms begin to appear when the damage reaches a point where the brain can no longer repair itself. The hallmark alterations or pathological characteristics linked to Alzheimer's disease include gliosis, amyloid plaques, neurofibrillary tangles, and extensive neuronal degeneration.

Presenilins

Intramembranous aspartyl proteases include presenilins. Nine transmembrane helix domains are present in both PSI and PSII. Transmembrane domains six and seven contain the aspartyl residues that exhibit catalytic activity (one on each domain). Between transmembrane six and seven, PSI and PSII go through proteolytic processing to create N-terminal and C-terminal fragments that stay connected to one another during complex formation. The motif that distinguishes PSI from PSII enables PSII to interact with AP-1 complexes and, consequently, endosome-lysosome complexes in a phosphorylation-dependent way. PSI has a larger spectrum of target proteins since it lacks this motif.

Figure 3: Progression of AD

Nicastrin

A type 1 integral membrane protein is called nicastrin. Its three structural domains are the transmembrane helical domain, the huge N-terminal extracellular ectodomain, and the C-terminal cytoplasmic domain. A conserved DYIGS motif found in the N-terminal domain aids in substrate selection. Only when substrates are cleaved by alpha or beta secretases is their N-terminal revealed. Nicastrin forms a scaffolding molecule with APH-1, which subsequently attaches to PEN-2 and PS1 or PS2. According to recent research, nicastrin is not required for gamma secretase action, despite its involvement in substrate recognition.

Anterior Pharynx-defective 1

The cytoplasmic C-terminal domain, the transmembrane segment, and the extracellular Nterminal area are the three domains of anterior pharynx-defective 1 (APH-1). It is believed that APH-1's interactions with other gamma secretase complex subunits are influenced by the conserved GXXXG motif present in the N-terminal region. Therefore, APH-1A and APH-1B are the two forms of APH-1 found in humans. The three paralogous genes that encode APH-1 in mice produce the three protein products known as APH-1A, APH-1B, and APH-1C. It's unclear what kind of APH-1 attaches to the PS1 or PS2 complex subunits.

Presenilin Enhancer 2

Three domains make up the 12K-Da protein called presenilin enhancer 2 (PEN-2): the transmembrane domain, the cytoplasmic domain at the C-terminus, and the extracellular domain at the N-terminus. There are three helical configurations in the transmembrane domain. PEN-2 primarily participates in the maturation and stabilization of the complex by binding to the fourth transmembrane region of presenilin-1.

Neurofibrillary Tangles

Tau is an axonal protein that stabilizes microtubules by binding to them via the MTBD. It speeds up tubulin polymerization, which facilitates microtubule assembly. Tau binding and detachment from microtubules are controlled by the phosphorylation and dephosphorylation cycles, respectively. The dynamic balance between tau phosphorylation and dephosphorylation is upset by mutations in APP or PS1 in AD, leading to abnormal phoshorylation and a decrease in tau's affinity for microtubules. The aberrantly phosphorylated tau begins to self-assemble into dimers, trimers, higher order oligomers, and protomers when it undergoes a conformational shift.

Gliosis

The process by which glial cells become activated in reaction to a stressor or challenge in the central nervous system is known as gliosis. Because post-mortem AD brains had higher amounts of proinflammatory cytokines and more activated microglia surrounding plaques, neuroinflammation is thought to be a major factor in AD pathogenesis. Glial cells (oligodendrocytes, microglia, and astrocytes) and cytokines that control inflammation are among the biological elements that are essential to the neuroimmune system. The development of AD is closely linked to immune system dysfunction. The interaction between the chemokine fractalkine (CX3CL1) and its receptor (CX3CL1R) decreases microglia's capacity for phagocytic activity, indicating a reduction in Aβ clearance. Reduced Aβ deposition was the outcome of microglia having insufficient amounts of the NLRP3 inflammasome. It has been demonstrated that increased IL-10 levels result in neuronal dysfunction and 35 losses. While IL-4 and TGF-β inhibit the proinflammatory response, IL-6 and TNF-α mitigate the pathophysiology of AD. Aβ clearance is linked to microglia. Increased aggregation is the outcome of decreased Aβ clearance from the brain caused by compromised microglial function and insufficient phagocytic activity. Additionally, astrocytes can exacerbate immunological reactions. But through the Aβ-degrading enzyme neprilysin, they can also help with Aβ clearance.

Gross Neuronal Loss

Neuron loss is one of the pathogenic characteristics of AD. It is well recognized that AD causes changes in hippocampal volume. AD patients' post-mortem brains exhibit enlarged ventricles and brain atrophy. This is mostly caused by neuronal death and degeneration.

Mild Cognitive Impairment

Cognitive tests are used by the doctor to make the diagnosis (Roberts and Knopman, 2013). In comparison to someone of his own age and educational level, the person exhibits diminished cognitive capacities. The most frequent alterations are in executive processes such as memory and attention. Distinguishing MCI caused by AD from MCI caused by other illnesses is challenging.

3. ALZHEIMER'S DISEASE DEMENTIA

When a person's cognitive abilities steadily decline to the point where they can no longer operate independently, they are frequently diagnosed with dementia. Delirium or other mental disorders are not the source of this cognitive impairment. Health history, family history, and cognitive tests like the MMSE or MoCA are used to diagnose dementia. A person must have at least two of the following deficits, according to the diagnostic criteria, in order to be diagnosed with dementia: altered behaviour or personality, diminished reasoning, decreased language function, impaired visuospatial abilities, or impaired memory for new knowledge. The diagnosis is dependent on biomarkers, the severity of symptoms, and the rate of advancement, and it can be difficult to differentiate AD from dementia.

Biomarkers are measurable components that can be used to determine whether or not a disease is present. Additionally, it can be utilized to ascertain the disease's stage. AD can be identified for a long time since neurological alterations start early in the disease. Better disease management will result from early intervention. Direct and indirect biomarkers are the two categories into which AD biomarkers fall. The pathophysiology of disease is represented via direct biomarkers. For instance, tau and Aβ in AD. Neuronal damage in general, rather than a particular disorder, is represented by indirect biomarkers. Aβ is the most widely used direct biomarker for AD. The CSF of AD patients has lower amounts of Aβ than those of healthy people. Atrophy in the medial, basal, and lateral temporal lobes and decreased absorption of 18-fluorodeoxyglucose (FDG) in the temporo-parietal cortex are other signs of neuronal degeneration.

Positron emission tomography (PET) and magnetic resonance imaging (MRI) are the two main imaging modalities used to investigate or diagnose AD. MRI is used to investigate changes in connectivity and gross anatomy. The distribution of tau and Aβ deposits is investigated using PET. The most conclusive diagnostic approach currently in use combines CSF markers with imaging methods. Long before AD symptoms appear, MRI has revealed structural and functional abnormalities in the brain. It has been demonstrated that cortical thinning is a predictor of cognitive decline. Another MRI-based marker for AD diagnosis is hippocampal volume.

Diffusion anisotropy-based magnetic resonance imaging (MRI) has revealed structural alterations in white matter, particularly in the corpus callosum, cingulum bundle, uncinate fasciculus, and frontal and temporal lobes. According to functional MRI research, AD patients' brain activity is altered during the resting state as compared to normal people. According to functional MRI research, AD patients' brain activity is altered during the resting state as compared to normal people.

3XTG

Three genes—APP, PS1, and MAPT—have mutations in the 3XTG model. The mutations include PSEN1-M146V, MAPT-P301L, and APP-KM670/671NL (Swedish). This model's genetic background is a hybrid between 129X/SvJ and 129S1/Sv. These mice exhibit neurofibrillary tangles in addition to plaques. While the tangled pathology is apparent by 12 months of life, the plaques begin to show as early as 6 months. By the time the child is 7 months old, there are more GFAP-positive cells, a sign of gliosis. By six months of age, synaptic dysfunctions including LTP impairment are evident. As early as four months of age, cognitive impairment manifests as retention-retrieval deficiencies.

APP23

The APP23 model, sometimes referred to as the Swedish model, is characterized by a single mutation in the APP gene (KM670/671NL). This model's genetic background is C57/BL6. The expression of APP is seven times higher in these mice. At six months of age, plaques are initially observed. In this model, neurofibrillary tangles are not present. By nine months of age, gliosis is evident. By 14 months of age, the hippocampal CA1 area has lost neurons. Beginning at three months of age, impaired spatial memory develops quickly. These models have not shown any changes in LTP/LTD till they are 12 months old.

APP/PS1

The Swedish mutation and the L166P mutation in PSEN1 are the two mutations found in APP/PS1 mice. The genetic background of this model is C57/BL6. The alterations are expressed at the Thy1 promoter, and APP expression is three times more. Around three months of life, plaque deposition begins in the hippocampal region, and by five months, it has extended to the cortex. At 4 weeks, there is a loss of spine, and at 17 months, there is a loss of neurons. In water maze trials, cognitive impairment is observed at 7 months of age. There are no tangles.

APPSwe/PSEN1dE9

Two mutations are present in these mice: a PSEN1 mutation (dE9) and a Swedish mutation. A C3:3 mouse with a Swedish mutation and hAPP expression was crossed with an S-9 mouse with a pSEN1 mutation to create the model. Around seven to eight months of age is when plaque deposition begins. Tangles and gliosis were not noticed until the child was 14 months old.

APP NL-F Knock-in

The APP NL-F Knock-in model was created to distinguish between the effects of excessive APP expression and those of excessive Aβ species production. Its two mutations are the Iberian mutation (APP I716" F") and the Swedish mutation (NL). These two mutations are incorporated into the humanized Aβ region and expressed under the mouse APP promoter. The Iberian mutation raises the ratio of Aβ42 to Aβ40, while the Swedish mutation causes overexpression of Aβ. This model's genetic background is C57/BL6. Deposition of plaque begins at six months of age. The investigations have not yet reported on tangles or 41 neuronal losses. At six months of age, activated GFAP-positive cells are visible around the plaques. Together with two other mouse strains, APP NL Knock-in and APP NL-G-F Knock-in, this model was created. There is only a Swedish mutation in APP NL Knock-in and no plaque pathology. Three mutations—Swedish, Iberian, and Arctic—are present in APP NL-G-F Knock-in, which has a more aggressive pathogenesis (APP E693G). As early as two months of age, gliosis and plaque accumulation can be noticed. There aren't any tangles and no evidence of neuronal loss yet. At six months of age, Y-maze tasks reveal memory impairment.

Aβ Related Treatment Approaches

The notion that Aβ species plays a significant role in the pathophysiology of AD is supported by several arguments: First off, the presence of amyloid plaques is one of the primary features of Alzheimer's disease. Second, synaptic toxicity caused by Aβ oligomers has been demonstrated. Thirdly, Aβ42 production is elevated in all the variants linked to early-onset AD. Fourth, amyloid plaque formations are associated with increased pro-inflammatory cytokines, activated microglia, and neuronal injury. Fifth, a higher Aβ load is linked to the risk allele APOE4. Researchers are now pursuing treatment approaches that target Aβ as a result of these findings.

Modulation of Aβ Production

Either α-secretase or β-secretase (BACE) can cleave the Aβ precursor molecule APP, and γ-secretase can then cleave it again. α-secretase cleavage is the non-amyloidogenic pathway since it produces Aβ40. The amyloidogenic pathway is caused by BACE cleavage, which produces Aβ42, which has a greater tendency to aggregate.

Inhibition of Aβ Aggregation

44 Oligomers can be formed by monomeric Aβ, namely Aβ42. Numerous investigations have demonstrated that these oligomers are neurotoxic. Oligomers develop into plaques. In addition to creating stress reactions around themselves, these aggregates are challenging to remove from the brain. Therefore, many groups have examined the strategy of preventing Aβ aggregation. The creation of compounds or peptides that can attach to monomeric fibrils and stop oligomerization has received a lot of attention. Successfully crossing the blood-brain barrier and getting the drug's concentration to approach ED50 in the brain are frequent issues that many of these molecule's encounter. Few inhibitors have advanced to clinical trials as a result of these crucial factors. One such chemical that shown effectiveness in Phase I and Phase II clinical studies as well as animal models was trimiprosate. The Phase III clinical trial was unsuccessful. Another such substance that did not pass a Phase II clinical trial was scyllo-inositol. Additionally, metal-protein attenuating substances—specifically, copper and zinc—are being investigated as possible inhibitors of Aβ aggregation. Clioquinol is one example of such a substance (Nguyen et al., 2021).

Enhancing Aβ Clearance

Proteases that break down Aβ and facilitate its removal from the brain include plasmin, insulin-degrading enzyme, and neprilysin. It is challenging to specifically activate these proteases. Aβ42 levels were markedly reduced by PAI-1 inhibitors in animal models of AD. Targeting Aβ transporters like as LRP and RAGE is an additional strategy. Although several RAGE inhibitors are undergoing clinical studies, none of them have demonstrated any noteworthy impact.

Immunotherapy

According to the first set of research on immunotherapy for AD, passive immunization with Aβ42 significantly reduces AD pathology in animal models and produces a highly specific immune response to remove Aβ. As a result, mice developed antibodies against human Aβ42. When these antibodies were administered to animal models, brain Aβ42 levels were significantly reduced, and cognitive scores improved. Table No. below lists the antibodies and agents that are undergoing clinical testing. The mechanism by which immunotherapeutic drugs work has been thoroughly examined. The agents appear to work primarily via activating microglia and phagocytosis.

Inflammation Related Approaches

Two facts serve as the primary foundation for the reasoning behind inflammation-related treatment approaches: first, recent genome-wide association studies have found a correlation between immune system-related genes, such as CR1, and AD; second, patients with arthritis who have been taking NSAIDs for a long time have a lower incidence of AD. Consequently, clinical trials were conducted to test a number of NSAIDs, including celecoxib, rofecoxib, and naproxen, however none of them demonstrated any therapeutic impact against AD.

4. EXISTING DRUGS FOR AD

46 Currently, symptomatic treatments are the medications given to treat AD. They delay the disease's course and alleviate behavioural and cognitive symptoms, but they cannot reverse the condition. Drug prescriptions differ depending on the patient's country of residency, related conditions, and the severity of the sickness. Drugs can be divided into two groups according to their modes of action (Godyń et al., 2016).

Lipoprotein Receptor Related Protein 1

Numerous proteinase inhibitors, which are typically present in plasma at high quantities, control blood protease activity. A proteinase and its inhibitor combine to produce a complex, which is subsequently removed from the bloodstream by the receptor system.

Withania Somnifera

Ashwagandha, or Withania somnifera in Sanskrit, is a plant in the Solanaceae family. Common names for it include Indian ginseng and winter cherry. The plant grows widely in drier regions of tropical and sub-tropical zones and can reach heights of 0.5 to 2 meters. Most of Africa, North America, India, China, and Australia are known to cultivate it. Ashwagandha is primarily grown for its therapeutic properties in India. It falls within the category of "medhyarasayana" formulations, which are treatments for the central nervous system. Medicinal formulations are made using either the entire plant or specific sections of it, such as the leaves, stem, or root (Cao et al., 2018). Additionally, it can be utilized either by itself or in conjunction with other therapeutic plants.

5. NOVEL TREATMENT APPROACHES

We created a reporter assay technique based on luciferase to screen the fractions and sub-fractions in vitro. This approach was justified by the idea that the luciferase gene's expression beneath the LRP promoter would provide an indirect readout of the fractions' activity on the promoter region. A fraction's luminescence will increase with its enhancer activity. This method also allowed us to address the question of where the enhancer target sites are on the LRP gene and examine the different LRP promoter regions independently. Selecting a promoterless vector containing the luciferase gene was the initial step in answering the aforementioned issues (Folch et al., 2016). Promega Corporation in Wisconsin, USA, provides pGL3-Basic, which we used as the vector. Its 4.8 kbp size comprises a luciferase gene, an ori site, a multiple cloning site (MCS), and the antibiotic resistance gene ampicillin.

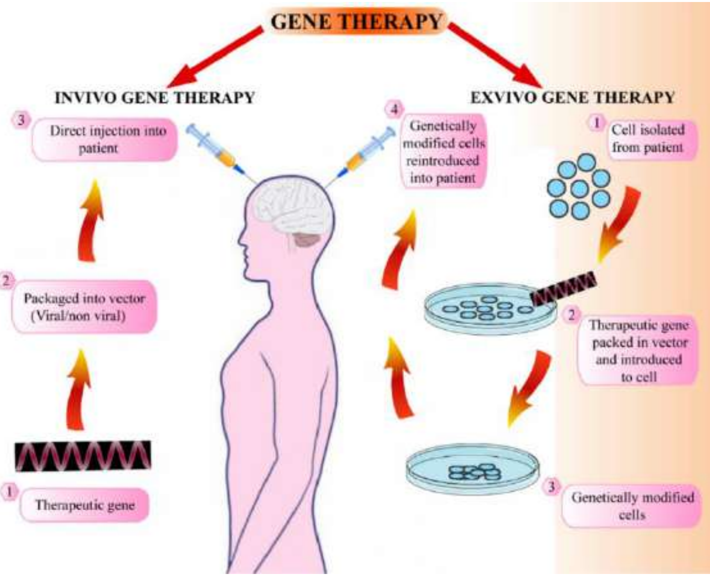

Figure 3: Gene Therapy

The first step in cloning a gene's promoter region into a vector is to separate the DNA that contains the gene. Creating forward and backward primers that will attach to the region of interest and aid in its amplification is the second stage. Following the creation of appropriate primers, the region of interest is amplified using the polymerase chain reaction (PCR). It is now necessary to ligate this amplified product into the vector. This is accomplished by digesting the vector and the amplified product using the same set of restriction enzymes, followed by ligation (Ju & Tam, 2022). To validate that the vector contains the appropriate promoter region, restriction digestion and PCR are utilized once more. The vector and insert are changed into competent bacterial cells after their identities have been verified. Following the inoculation of these cells into LB broth, the vector containing the insert is cloned. From these cells, the plasmid DNA is subsequently extracted. By using PCR or restriction digestion, a tiny quantity of plasmid can be utilized to verify the clone's identification once more. The next sections provide a detailed description of the fundamental idea, necessary supplies, and steps for each of the aforementioned processes.

The Qiagen DNeasy Tissue Kit's DNA extraction process is founded on the ideas of spin-column-based nucleic acid purification. A lysis reagent made up of chaotropic chemicals is used to lyse the cells open. The cells release their nucleic acids. The samples are then combined with a buffer solution that contains either isopropanol or ethanol. The binding solution is this. At the proper pH, it makes nucleic acid binding to the silica column easier. The bound nucleic acid is subsequently released with the help of elution buffer, allowing it to be collected in a vial.

Marion M. Bradford created the Bradford Assay in 1976. Protein concentration is determined using a quantitative spectroscopic technique. It is predicated on the idea that arginine, lysine, and histidine are basic amino acids that, when bound by Coomassie blue dye, form a blue-colored complex under basic circumstances. The intensity of color produced increases with protein concentration. The dye comes in three varieties: anionic (blue), neutral (green), and cationic (red).

Antigen-antibody interaction is the fundamental idea behind ELISA. An enzyme and a specific antibody are used to incubate the antigen, which is a material with an unknown concentration. When a chromogenic substrate is present, this enzyme aids in the creation of color. The 88 pure or recombinant antigens used to generate the standards are whose concentration in the sample needs to be ascertained.

TRI reagent and bromochlorophenol (BCP) were used to isolate RNA. Phenol and guanidine isothiocyanate are present in a monophasic solution in the TRI reagent. Protein denaturation, RNase inactivation, and rRNA separation from ribosomes are all caused by guanidium, thiocyanate, and phenol. Additionally, phenol aids in the separation of DNA and RNA. Additionally, BCP denaturates proteins. By eliminating lipids and separating monophase into three different phases—the organic phase, interphase, and aqueous phase—it facilitates improved RNA extraction. Protein is found in the organic phase, whereas DNA is found in the interphase. After being extracted, RNA is placed in an aqueous phase that can be separated and subsequently precipitated with ethanol.

Single-stranded cDNA was prepared using Applied Biosystems' High-Capacity cDNA Reverse Transcription Kit. Single-stranded complementary DNA is produced from RNA by reverse transcriptase. The kit's instructions were followed in order to prepare the reaction mixture. The reagents utilized are as follows: 0.4 ul of 25X 100mM dNTP mix, 1 ul of 10X reverse transcription buffer, 1 ug of RNA, 0.5 ul of reverse transcriptase, and 1 ul of 10X random hexamer primers.

With real-time PCR, the PCR product may be precisely quantified at each cycle. Fluorescent reporter dyes added to the product are used to track the PCR reaction in real time throughout the exponential phase. DNA binding dyes, fluorescent primers, and fluorescent probes are examples of fluorescent reporters that are frequently utilized. When SYBR green attaches to double-stranded DNA (dsDNA), it fluoresces, and the quantity of dsDNA present determines how strong the fluorescent signal is. The number of amplicons produced during the exponential phase is directly correlated with the rise in the fluorescent signal. This makes it possible to monitor the PCR product accumulation. Fluorophores are linked to certain primers in primer-based fluorescent detectors; as the primers are integrated into the PCR product, the fluorescence rises, signifying an increase in the number of amplicons (Wolfe, 2002).

Proteins on polyacrylamide support migrate in an electric field with a net negative charge. Proteins can be quantified and described using a technique called sodium dodecyl sulphate-polyacrylamide gel electrophoresis, or SDS-PAGE.

Since the LRP promoter area has not been thoroughly investigated, little is known about the transcription factors and other regulatory components linked to LRP overexpression. Determining how well various promoter regions drive the production of LRP when treated with the extract is one method to answer this question (in the context of the current investigation). We could identify the transcription factors and co-activators linked to the overexpression of LRP by identifying the active promoter area. The next part provides a description of the experiment's findings. Additionally, we chose to use just the 400bp clone for all subsequent tests because the activity of fractions and WE was similar among the three clones.

6. DISCUSSION

About 35 million people worldwide suffer with Alzheimer's disease (AD), a progressive neurological illness. There is currently no cure for AD, despite the fact that the genetic abnormalities linked to the disease were discovered more than 20 years ago. Only symptomatic treatment is offered by commercially accessible medications like donepezil and memantine. The incidence of AD patients is rising annually due to an increase in average life expectancy, making the development of more effective therapeutic drugs that can change the course of the illness essential.

Traditional medical systems like Ayurveda and medicinal plants have received a lot of interest as a result of the desire to develop better therapeutic tactics and identify better therapeutic targets. More than 200 WS root formulations have been documented in traditional Indian medical systems; the most of these were used to treat anxiety, tension, and memory problems. According to earlier research from our lab, a semipurified extract of Withania somnifera root cures plaque pathology and behavioral abnormalities in nine-month-old APPSwe/PS1dE9 rats by upregulating hepatic LRP (Sehgal et al, 2012). Amyloid beta 42 (Aβ42) is sucked up by LRP1 in its soluble form (sLRP). Therefore, higher plasma levels of sLRP lead to higher plasma clearance of Aβ42. This causes Aβ42 monomers to flow from the brain into the periphery. The oligomers begin breaking down into monomers as soon as the monomers are eliminated from the brain, and plaque destruction also starts (Barage & Sonawane, 2015; Graham et al., 2017; Pinheiro & Faustino, 2019; Nalivaeva & Turner, 2019; Gao et al., 2018).

LRP expression has also been linked to nuclear respiratory factor 1 (NRF1). The NRF1 element, also known as the NRF1 binding sequence, was located at -143 base pairs and contained intrinsic sites for the binding of interferon gamma (IFN gamma) and cyclic adenosine monophosphate (cAMP).

Circadian rhythms are essential to almost every type of life. The SCN in mammals synchronizes autonomous cellular clocks to produce a complex rhythm of energetics and metabolism that results in organism-level behaviour. Our goal was to determine how continuous light affected the pathophysiology of AD (Zhang et al., 2021; Derakhshankhah et al., 2020; Oualla et al., 2017; Perricone & Vander Heide, 2014; Jain & Jain, 2013).

However, in at-risk AD rats, circadian disturbance facilitated the aggregation of exogenously given Aβ42. While rifampicin simply stopped plaque from forming and had no effect on cognitive symptoms or oxidative brain damage, daily fluoxetine medication totally restores animals' circadian rhythms and rids them of AD-like pathology. Additionally, the levels of circadian rhythm markers Per2, PRX1, and PRX-SO2/3 in the SCN were disturbed after four months of continuous light. Apart from the memory impairment observed in animals exposed to continuous, long-term light, the neurobiochemical analyses verified that AD pathology is in progress, as demonstrated by a notable increase in endogenous Aβ42 levels and dysregulation of several genes related to AD, including Bace1, Sirt1, Prokr2, and Mgat3.

7. CONCLUSION

Alzheimer's disease (AD), a degenerative neurological condition, is characterized by cognitive and decision-making difficulties in addition to memory loss. Even though the first case of AD was reported more than a century ago, there is still no treatment for it. The commercially available medications, such as donepezil and memantine, only partially alleviate symptoms. They neither stop the disease's progression nor treat it. Therefore, it is essential to develop novel therapeutic approaches and targets.

The results demonstrate that Wistar rats who are exposed to continuous light for an extended period of time develop behavioural abnormalities and brain oxidative damage. Additionally, prolonged exposure to light triggers a number of upsetting molecular processes that lead to a phenotype resembling AD. By restoring circadian regularity, fluoxetine therapy saved rats against an AD-like phenotype. We suggest that shift workers may be more susceptible to AD, and that symptoms may manifest earlier in individuals who are predisposed, making it more difficult to administer the right treatment on time.

Even though Aβ deposition form toxic aggregates is believed to be the hallmark of AD, dementia and cognitive impairment cannot be caused by this alone. The accumulation of p-tau, which is connected to neuronal dysfunction and death, is more strongly associated with the onset of cognitive decline. Neuroinflammation may have two functions: it can be advantageous when it promotes the removal of Aβ and protects against damage, and it can be harmful when it is continuously triggered, feeding a number of pro-inflammatory pathways linked to neurodegeneration. Relying exclusively on misfolded protein deposition may only be a partially successful strategy. Nonetheless, it is crucial to identify neurodegenerative alterations early on at a potentially curable stage of cognitive decline. One of the most significant issues with treating AD that researchers have faced in the last 20 years is determining the appropriate targets at the appropriate time. A multimodal approach that incorporates anti-amyloid and tau therapies with the management of neuroinflammatory reactions may work well in this field.

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