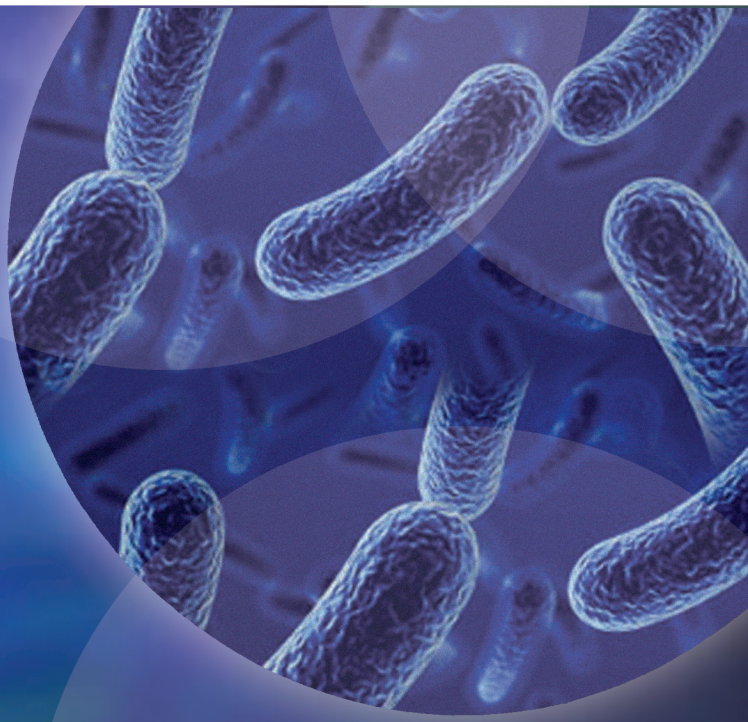
Frontiers in Clinical Drug Research

(Anti Infectives)



Editor:
Atta-ur-Rahman, *FRS*

Bentham Books

Frontiers in Clinical Drug Research-Anti Infectives

(Volume 8)

Edited by

Atta-ur-Rahman, *FRS*
Honorary Life Fellow,
Kings College,
University of Cambridge,
Cambridge,
UK

Frontiers in Clinical Drug Research-Anti Infectives

Volume # 8

Editor: Prof. Atta-ur-Rahman, *FRS*

ISSN (Online): 2352-3212

ISSN (Print): 2452-3208

ISBN (Online): 978-981-5039-41-2

ISBN (Print): 978-981-5039-42-9

ISBN (Paperback): 978-981-5039-43-6

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE

The 8th volume of **Frontiers in Clinical Drug Research – Anti Infectives** comprises seven chapters that cover several important topics including the role of monoclonal antibodies, emerging antiviral agents, and the current situation on antiparasitic drug discovery.

In chapter 1, Capela *et al.* discuss the role of monoclonal antibodies as therapeutic agents for inflammatory diseases. Doshi *et al.* explore the pharmacotherapy of emerging antiviral agents in chapter 2 of the volume. Al-Azzawi and Sakr discuss how vitamin D can be used as a preventive or treatment agent for COVID-19. Das *et al.,* in chapter 4, present an overview of anti-infectives to combat leishmaniasis. Rayes *et al.,* in chapter 5, summarize the current situation on antiparasitic drug discovery and discuss the use of *C. elegans* at the initial steps of drug development. In the next chapter, Kannan *et al.* discuss the emergence of the Zika virus and its detailed genome structure and replication cycle. Fuselli *et al*., in the last chapter of the volume, give an insight on agro-industrial waste, a new source of raw material for the control of American foulbrood in honey bees.

I would like to thank all the authors for their excellent contributions that should be of great interest. I would also like to thank the editorial staff of Bentham Science Publishers, particularly Mr. Mahmood Alam (Editorial Director of Bentham Science Publishers), Mr. Obaid Sadiq (In-charge Books Department), and Miss Asma Ahmed (Senior Manager Publications) for their support.

Atta-ur-Rahman, *FRS*
Kings College
University of Cambridge
Cambridge
UK

List of Contributors

Ami P Thakkar	Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India
Azucena Iglesias	Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP, Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
Ana P.M. Tavares	CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
Anindita Ukil	Department of Biochemistry, University of Calcutta, Kolkata 700019, India
Balaji Gowrivel Vijayakumar	Department of Chemistry, Pondicherry University, Kalapet, Puducherry, 605014, India
Cristina Ramírez	Depto. de Química y Bioquímica, FCEyN, Universidad Nacional de Mar del Plata, Argentina
Deepthi Ramesh	Department of Chemistry, Pondicherry University, Kalapet, Puducherry, 605014, India
Diego Rayes	Laboratorio de Neurobiología de Invertebrados, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) CCT UNS-CONICET. Bahía Blanca, Argentina, Camino La Carrindanga Km 7, Bahía Blanca, Argentina Dpto de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. Bahía Blanca, Argentina, San Juan 670, Bahía Blanca, Argentina
Emanuel V. Capela	CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
Florencia Fangio	Departamento de Química, Facultad de Ciencias Exactas y Naturales, Instituto de Investigaciones Físicas de Mar del Plata (IFIMAR), CONICET, Universidad Nacional de Mar del Plata, Mar del Plata, Buenos Aires, Argentina
Gaurav M Doshi	Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India
Hemen S Ved	Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India
Jéssica Bairos	CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
Mara G. Freire	CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
Mahmood A. Al-Azzawi	Department of Pathological Analysis Techniques, College of Medical & Health Technologies Ahl Al Bayt University, Karbala, Iraq

María Gabriela Blanco	Laboratorio de Neurobiología de Invertebrados, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) CCT UNS-CONICET. Bahía Blanca, Argentina, Camino La Carrindanga Km 7, Bahía Blanca, Argentina Dpto de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. Bahía Blanca, Argentina, San Juan 670, Bahía Blanca, Argentina
María José De Rosa	Laboratorio de Neurobiología de Invertebrados, Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) CCT UNS-CONICET. Bahía Blanca, Argentina, Camino La Carrindanga Km 7, Bahía Blanca, Argentina Dpto de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. Bahía Blanca, Argentina, San Juan 670, Bahía Blanca, Argentina
Matias Maggi	Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP, Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
Moustafa A. Sakr	Molecular Diagnostics and Therapeutics Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt
Nirav Y. Bhatia	Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India
Pablo Giménez-Martínez	Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP, Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
Prachi M Pimple	Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India
Pijush K. Das	Infectious Diseases and Immunology Division, CSIR- Indian, Institute of Chemical Biology, Kol-kata 700032, India
Shalini Roy	Department of Biochemistry, University of Calcutta, Kolkata 700019, India
Sandra Churio	Departamento de Química, Facultad de Ciencias Exactas y Naturales, Instituto de Investigaciones Físicas de Mar del Plata (IFIMAR), CONICET, Universidad Nacional de Mar del Plata, Mar del Plata, Buenos Aires, Argentina
Sandra Fuselli	Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP, Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina Comisión de Investigaciones Científicas (CIC), Buenos Aires, Argentina
Tharanikkarasu Kannan	Department of Chemistry, Pondicherry University, Kalapet, Puducherry, 605014, India

CHAPTER 1

Monoclonal Antibodies as Therapeutic Agents for Inflammatory Diseases

Jéssica Bairos[1,$], Emanuel V. Capela[1,$,*], Ana P.M. Tavares[1] and **Mara G. Freire[1]**

[1] *CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

Abstract: Inflammation is a physiological process caused when an agent (chemical, biological or physical) transcends the primary defense barrier of an organism, setting a series of biological reactions to restore the integrity of such organism, thus playing a central role in the fight against those pathogens. Uncontrolled amplification of these events may lead to undesirable pathological manifestations such as cancer, diabetes, and cardiovascular, neurological, and chronic inflammatory diseases. Monoclonal antibodies (mAbs) were first described in 1975, and since then, they have proven to be relevant therapeutic agents in a myriad of diseases. The US Food and Drug Administration (FDA) has already approved more than 90 mAbs for the treatment of several diseases, from which approximately 26% were specifically approved for the treatment of inflammatory diseases, for instance, rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, and palmoplantar pustulosis. This chapter provides an overview of the inflammation process and main biochemical mechanisms, together with a vision on the current state of the art of the mAbs-based biopharmaceuticals market and their application as powerful therapeutic agents for inflammatory diseases.

Keywords: Biopharmaceuticals, Biopharmaceuticals Market, Biochemical Mechanisms, Inflammation, Inflammatory Diseases, Monoclonal Antibodies, Therapeutic Agents.

INTRODUCTION

Inflammation consists of the natural protective response of the body to injury. It occurs when an agent (chemical, physical or biological) transcends the primary defense barrier of the organism [1, 2]. It plays a central role in the fight against pathogens and can set biochemical reactions to restore homeostasis through the

* **Corresponding author Emanuel V. Capela;** CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal; Tel: +351-234-401422, Fax: +351-234-370084;
E-mail: emanuelcapela@ua.pt
$ Equal contribution

Atta-ur-Rahman (Ed.)

activation of specific components, which act through the destruction or isolation of the aggressor agent [3, 4]. Inflammation can be manifested as an acute process comprising three main events: i) increased blood flow, ii) development of edema, and iii) migration of leukocytes to the inflammatory focus [2]. Uncontrolled amplification of these events may lead to a chronic process, which is long-term and associated with the presence of lymphocytes fibrosis and tissue necrosis [5, 6]. This phenomenon causes undesirable pathological manifestations such as cancer, diabetes, and cardiovascular, neurological, and chronic inflammatory diseases [5, 6]. Therefore, this type of diseases' progression fostered the search for effective alternative therapies, which is a crucial objective to be achieved in the coming years.

In recent decades, technological advances in bioprocess engineering have increased the interest in the development of alternative therapies for inflammation treatment, particularly recurring to biopharmaceuticals [7]. Biopharmaceuticals are biological macromolecules or cellular components that can be used in vaccines or as therapeutic agents. They are obtained by biological processes (*in vitro* or *in vivo*) and are extracted from biological sources, for example, tissues and organs, microorganisms, fluids of animals, mammalian cell cultures, insects, and also plants [7]. The main examples include recombinant proteins (*e.g.,* monoclonal antibodies) and nucleic-acid-based products, which can be applied in the treatment of several inflammatory diseases, such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriasis, psoriatic arthritis, and palmoplantar pustulosis [8]. Among them, monoclonal antibodies are the most used biopharmaceuticals, representing 53% of all biopharmaceuticals approved [9].

Monoclonal antibodies (mAbs) were firstly described by Köhler and Milstein in 1975 [10], and since then, they have become the new backbone of the pharmaceutical industry as they have exquisite target selectivity and specificity [10]. mAbs offer the most promising prospects for new therapeutic approaches for inflammatory diseases [11, 12]. The most successful applications of mAbs are in autoimmune and inflammatory conditions [13]. Furthermore, as a wide range of mAb-based agents targets several cytokines, chemokines, adhesion molecules, receptors, and various types of cells, it is expected that these therapeutic "magic bullets" [14] will greatly expand in the future while providing better personalized treatment for a wide range of diseases. In this chapter, the most important aspects and main biochemical mechanisms of the inflammation process are overviewed, followed by a current review on the mAbs-based biopharmaceuticals market and approved mAbs product/therapies for inflammatory diseases. The action mechanisms and features of some relevant mAbs are also discussed, highlighting the advantages of mAbs-based therapies, together with the steps required for their increased adoption and widespread use.

MONOCLONAL ANTIBODIES

Structure and Properties of Antibodies

Antibodies, usually referred to as immunoglobulins (Igs), are glycoproteins found in plasma and extracellular fluids [15]. They are essential biomolecules in the immune-humoral system of all vertebrates [15], being the line of defense of the immune system [16]. They are produced naturally by specific plasma cells, namely the B lymphocytes, in response to exposure to "foreign" molecules or other antigens [15, 16]. In their composition, they present one or more regions, the paratopes, which recognize and bind to the epitopes of the antigen. This molecular recognition allows the neutralization and/or elimination of the antigen, allowing the organism to protect itself against the action of microorganisms and other harmful species, such as foreign proteins [17 - 19], carbohydrates [19], peptides [17], bacteria [17, 19], viruses [17], fungus [18] or even cancer cells [18]. As a result, an effective immune response takes place, often involving the production of a vast array of antibodies that are structurally similar yet unique, thus enabling the multiple epitope binding onto a given antigen [17, 20].

Independently of their specificity, all antibodies are heterodimer proteins structurally composed with four polypeptides chains – two identical heavy chains (H) and two similar light chains (L), in a "Y"-like shape form (Fig. **1**) [15]. Disulphide bonds and non-covalent bonds are held together with chains by the "hinge" region, which provides stability and flexibility to the antibody. Furthermore, all polypeptide chains contain variable regions (V), presenting considerable variations in their amino-acid composition and where the antigen binds [17]. The constant regions (C), located at the carboxyl-terminal region, are specific for effector functions [17]. The antibody chains are further divided into L and H sections – each L chain has a variable domain (VL) and a constant domain (CL), while each H chain has one variable domain (VH) and three constants domains (CH1, CH2, CH3) [15, 17, 21], as shown in Fig. (**1**).

The antibodies may undergo proteolytic digestion, giving rise to new antibody fragments – Fab (**F**ragment **a**ntigen **b**inding) and Fc (**F**ragment **c**rystalline) [15]. Some enzymes can be highlighted in this topic: papain, for example, digests the antibodies into two Fab fragments and one Fc fragment, whilst pepsin cleaves the antibody below the disulphide bridge, generating an Fc fragment and a divalent F(ab') fragment [15]. The Fab fragment of the antibody contains the specific antigen-binding domain, and the Fc fragment is responsible for the effector properties, such as activation of natural killer cells, activation of the classical pathway of the complement system, and phagocytosis of the antigen [15].

CHAPTER 2

Pharmacotherapy of Emerging Antiviral Agents

Gaurav M Doshi[1,*], Hemen S Ved[1], Ami P Thakkar[1], Nirav Y. Bhatia[1] and **Prachi M Pimple[1]**

[1] *Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India*

Abstract: Anti-infective agents have been one of the greatest accomplishments of modern medicine, which has led to a decrease in the number of deaths caused by various infectious diseases. The anti-infective agents are a broad family consisting of antimicrobials, antifungals, antimalarials, antiprotozoal, antituberculosis, and antiviral agents. Viral infections have caused millions of casualties worldwide, leading to the need for the development of effective antiviral agents. Although the replication mechanism differs significantly between the viruses, all viruses undergo steps like attachment, entry, genome replication, gene expression, and assembly for the release of the virions into the body of the host. Treatment with antiviral agents is essential for blocking the replication of the virus, and the currently available antiviral therapies are directed according to the disease. Furthermore, the treatment with antiviral agents aims to eradicate the viral pathogen from the host and prevent the clinical manifestation. Infectious diseases, such as human immunodeficiency virus (HIV), hepatitis B, and hepatitis C virus (HBV and HCV), and influenza, are of significant global concern. On the contrary, the outbreak of newer strains of influenza virus and Zika virus, Ebola virus, strains of coronavirus (CoV) like severe acute respiratory syndrome (SARS – CoV), Middle East respiratory syndrome (MERS – CoV) and novel Coronavirus (2019-nCoV) are life-threatening viral infections that exhibit major challenges to the humanity. As of date, multiple effective virostatics that target specific viral replication steps are approved for the treatment of viral infections. However, the use of such agents is restricted given the rapid emergence of antiviral resistance, which remains a major concern of current antiviral therapy. In this chapter, we summarize recent antiviral agents that show promising clinical benefits in various phases of clinical trials and also consider them as potential therapeutic agents in the future. Besides, we highlight and analyze the development of novel inhibitors targeting various stages of the viral life cycle that act by distinct mechanisms against current and emerging viral infections. Many antiviral drugs currently available are based on the concept of traditional chemotherapy. Nevertheless, new developments and advances in molecular biology have opened up possibilities to alternate treatment approaches. Clinical trials to evaluate gene silencing mediated by small interfering RNA (siRNA) and antisense RNAs expression against infection with a respiratory syncytial virus (RSV) have recently been initiated. Moreover, *in–vitro* studies of antisense RNA or siRNA

[*] **Corresponding author Gaurav Doshi:** Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V. M. Road, Vile Parle (W), Mumbai, India;
Tel: 9819771515; E-mail: gaurav.doshi@bncp.ac.in

Atta-ur-Rahman (Ed.)

technology have shown promising results in various virus strains. Despite the recent advancements, the development of targeted delivery of antiviral RNA molecules remains a major challenge since DNA viruses and retroviruses can incorporate their genomes into human genomes. To emphasize, antiviral drugs against particular target proteins have been effective in the treatment of prevalent infectious diseases such as HIV and HCV. Thereupon, broad-spectrum antiviral drugs instead of antivirals against specific virus infections need to be designed. With the rapid development of in-silico tools and gene modification strategies, antiviral drugs with better therapeutic index and safety profile will be developed against infectious diseases in the future. In fact, the effective design of newer antiviral drugs will reduce the possibility of emerging antiviral resistance.

Keywords: Antivirals, Clinical trials, Coronavirus, Human immunodeficiency virus, Middle East respiratory syndrome, Pharmacotherapy, Respiratory syncytial virus.

INTRODUCTION

Viral infectious diseases are of great concern as they cause significant morbidity and mortality in humans. To combat viral diseases, it is essential to constantly develop antiviral drugs and vaccines. Around 90 antiviral drugs have been approved in the treatment of the nine viruses that cause human infectious diseases, such as human immunodeficiency virus (HIV), human cytomegalovirus (CMV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), influenza virus, respiratory syncytial virus (RSV), varicella-zoster virus (VZV) and human papillomavirus (HPV) [1, 2]. On the contrary, emerging and re-emerging viruses like Zika virus, Ebola virus, strains of coronavirus (CoV) like severe acute respiratory syndrome (SARS – CoV), Middle East respiratory syndrome (MERS – CoV), and novel coronavirus (2019-nCoV) exhibit major challenges to the humanity [3]. There is always a threat to the global health posed by drug-resistant virus strains, which can emerge because of the high mutation rates of RNA viruses. The primary focus in the past has been on virus targets, and this has proven to be a very effective approach. It is now being replaced by a different approach such that the strategies include compounds that target generic viral targets, such as RNA or DNA synthesis, which may be active against various viruses, as well as compounds that target host cellular activities needed for virus replication [4, 5].

Notably, toxic side effects limit the clinical use of currently approved antiviral drugs. In addition, antiviral chemotherapy has many specific drawbacks [6]. To begin with, selective antiviral drugs have a limited antiviral activity spectrum. Additionally, since antiviral drugs target the steps in viral replication, they are ineffective in the latent phases of certain viral infections. As a result, eradicating latent virus infections is currently not possible. Besides, antiviral treatment should

be started early to avoid permanent tissue damage. Timely treatment is highly unlikely without an early and precise diagnosis, which is difficult in the cases of many viral infections [1, 7]. Cross-resistance among antiviral drugs is the next major problem that must be addressed. Resistance to one drug is often associated with decreased susceptibility to another drug of the same class [8, 9]. Several approaches have been proposed to reduce resistance, including the use of combination therapy wherever possible, avoiding repeated use of antiviral drugs, and discontinuing the treatment whenever possible [10].

In this chapter, we give an overview of the major classes of antiviral drugs along with the development of novel inhibitors against current and emerging viral infections. To give a comprehensive overview, we highlight the most recent advances on antiviral drugs and their clinical perspectives against emerging infectious diseases.

EXISTING ANTIVIRAL CLASSIFICATION

5-substituted 2′-deoxyuridine Analogs

Many 5-substituted derivatives of 2'-deoxyuridine are used in the treatment of herpes virus-induced infections, whereas some derivatives inhibit the reproduction of the Poxviridae family viruses. Antiviral drugs like idoxuridine (5-iodo--'-deoxyuridine), trifluridine (5-Trifluoromethyl-2'-deoxyuridine), and brivudine (5-(2-bromoethenyl)-2'-deoxyuridine) are a class of 5-substituted 2′-deoxyuridine analogues [11, 12]. Although these drugs fall under the same group, the mechanism of their action differs. Idoxuridine, trifluridine, and brivudine are chemically similar to thymidine that inhibits the replication of various DNA viruses, mainly herpesviruses and poxviruses [13, 14]. In turn, the phosphorylated derivatives interfere with various enzyme systems. The triphosphate prevents the synthesis of viral DNA and is incorporated into viral and cellular DNA. Likewise, brivudine is converted by thymidine kinases of HSV-1 and VZV to its monophosphorylated form and then by cellular kinases into diphosphorylated and triphosphorylated forms. The triphosphorylated form gets incorporated into the viral DNA, which, in turn, blocks the action of DNA polymerases and inhibits viral replication. Serious side effects associated with thymidine analogs are transient burning or stinging and corneal defects. Unlike idoxuridine and trifluridine, brivudine is less toxic and has a favorable safety profile [15 - 17].

Nucleoside Analogs

Nucleoside analogs are used in the treatment of various infections caused by HSV, HCMV, VZV, HIV-1, HBV, and HCV due to their high antiviral potency and significant pharmacokinetic parameters. Vidarabine (9- (β--

CHAPTER 3

Antiviral Activity of Vitamin D and COVID 19: Current Understanding

Mahmood A. Al-Azzawi[1,*] and **Moustafa A. Sakr**[2]

[1] *Department of Pathological Analysis Techniques, College of Medical & Health Technologies, Ahl Al Bayt University, Karbala, Iraq*

[2] *Molecular Diagnostics and Therapeutics Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt*

Abstract: Innate and adaptive immune responses, which are intimately related to the evolution of many infectious diseases, are influenced by the biologically active form of vitamin D. From a mechanical perspective, there are several rationales to assume that vitamin D positively modifies host responses to SARS-CoV-2, either in the early infection or subsequent hyper-inflammatory stages of COVID-19. It has been long known that vitamin D metabolites induce antiviral effects through indirect and direct mechanisms *via* antimicrobial peptides, immune modulation, the interaction between major viral and cellular particles, initiation of apoptosis and autophagy, and diversity of hereditary and epigenetic aspects. The remarkable overlap between the deficiency of vitamin D and risk factors for severe COVID-19, including obesity, aging, and Black or Asian ethnicity, has motivated researchers to assume that supplementation of vitamin D can be promising as a preventive or treatment agent for COVID-19. Since the outset of the pandemic, researchers have integrated literature searches and cross-sectional statistical studies to appraise the vitamin D level impact of COVID-19, whereby nearly 30 observational studies have confirmed that the incidence, severity, and mortality of COVID-19 are inversely related to the serum 25OHD concentrations. Also, some recently announced clinical trials indicated that vitamin D supplementation has a positive effect on the severity of COVID-19; however, other studies, including clinical trials, have not supported that, especially if we take into account what was revealed in a recent clinical trial, *i.e*., airway diseases are related to the irregular metabolism of vitamin D increasing the potential of developing vitamin D deficiency due to pulmonary inflammation. Therefore, more dedicated studies are required without critical limitations to ascertain the actual effect of vitamin D in preventing and treating COVID-19, and if its effectiveness is proven, the effective dose must be determined.

[*] **Corresponding author Dr. Mahmood A.Al-Azzawi:** Department of Pathological Analysis Techniques, College of Medical & Health Technologies, Ahl Al Bayt University, Karbala, Iraq;
Tel: 009647826002311; E-mail: mmahmood41@yahoo.com

Atta-ur-Rahman (Ed.)

Keywords: ACE2, ARDS, Antiviral, Betacoronavirus, Calcidiol, Calcitriol, Coronavirus-2, COVID-19, Epidemiological, Ethnicity, Inflammation, Innate, Mortality, Pandemic, RAS, RCTs, SARS-CoV-2, Severity, Vitamin D.

INTRODUCTION

In the past twenty years, seven coronaviruses have appeared, causing mild to severe respiratory diseases in humans, including Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) (a Betacoronavirus lineage B/Sarbecovirus). These were responsible for causing respiratory syndromes that may sometimes be very severe and may cause multi-organ failure with reverse myocardial remodeling, myocardial stress, and cardiomyopathy, which can be fatal, especially among children, elderly, patients suffering from chronic lung and hypertension diseases, and those who live in cities with high pollution levels [1 - 4]. In late 2019, the world witnessed the third prime epidemic of Coronavirus (CoV) infection, originating from Wuhan, China, and it was first called 2019-nCoV [1], and then it was renamed by the World Health Organization to COVID-19 in February, 2020. A novel coronavirus (SARS-CoV-2) is recognized as the causative agent of Coronavirus Disease 2019 (COVID-19), which represents one of the most critical epidemiological events that have represented the greatest threat to public health in the past hundred years [5]. In addition to the worsening of the socio-economic conditions caused by this pandemic, it paralyzed the economy and stalled life in most parts of the world [6, 7].

Coronaviruses are positive-strand RNA viruses and enveloped in a lipid bilayer with ca 30 kb in size. These viruses infect a wide range of birds and mammals and are usually carried through the respiratory pathway [8, 9]. Four major coronaviruses (NL63, OC43, 229E, and HKU1) and three extremely pathogenic zoonotic (SARS-CoV, MERS, and SARS-CoV-2) are categorized in humans. Severe atypical pneumonia is usually the immediate cause of death [10, 11]. Unfortunately, none of them has efficient anti-viral drugs [12 - 15], hence there is an urgent need for effective treatments. Seven vaccines are currently administered with more than 75% efficacy.

The pathophysiology of COVID-19 disease and the major reason for death is due to the increased inflammatory molecules (accompanied by immune cell infiltration, necrosis, and infected tissues hyperplasia), mainly at the lung level; this impairs the pulmonary oxygen interchange, causing severe pneumonia [16 - 18]. Several systemic alterations have also been perceived, especially in patients over the age of 60, which involve perturbation of normal levels of plasma of lactate dehydrogenase enzyme, C-reactive protein, platelet cells, and lymphocytes [19]. Interestingly, the elderly were more prone to die [20]. While indicators of

the prevalence of the virus and its severity are increasing with its arrival to new geographical zones, clinical trials are being conducted for some hopeful vaccines, taking into consideration that it may not take less time to obtain data on the extent of their efficacy and levelsof antibody protection [7, 21 - 23]. Thus, the existing options to confront COVID-19 disease and epidemics presently are based on (1) implementing a wide range of antivirals that can alleviate virus infection, (2) mitigation of acute inflammatory clinical symptoms, and (3) social distancing between the population, taking into account the precautionary measures in cases of necessary socialization to limit the increase in infections [21, 24]. In the aspect of the lack of a definite treatment against the COVID-19 deadly pandemic, its rapid development, and the direct effects of social isolation on the economic situation, particularly in developing countries, the necessity emerges to search about the existing and known pharmacological agents that boost or augment the performance of the immune cells.

SARS-CoV-2 appears to essentially use the alveoli macrophage escape mechanism, followed by a cytokine storm and hyper inflammatory molecules in critical patients [7], as a pathogenic process for the development of acute respiratory distress syndrome (ARDS) [25]. The spike protein of SARS-CoV-2 binds angiotensin-converting enzyme 2 (ACE2) of the pneumocystis type II cell receptor [26]. Patients with COVID-19 showed significantly elevated plasma angiotensin-2 levels, which were directly proportional to the viral load copies and severe lung injuries [27]. Thus, this indicates a strong link between the COVID-19 disease and the Renin-Angiotensin System (RAS). It is possible for SARS-CoV-2 to target both upper and lower lung host cells and start the infection by the attachment of the spike (S) protein to the receptor of ACE2 [28 - 32]. This receptor is a crucial enzyme for controlling the RAS that adjusts blood pressure and blood vessel homeostasis. Interestingly, it was found that patients with high blood pressure, coronary heart disease, diabetes, and cerebral vascular disease exhibit higher ACE2 mRNA expression, and this may co-relate the higher risk of deadly COVID-19 disease in these individuals [33, 34]. In addition, SARS-CoV-2 can also target the central nervous system cells *via* ACE2 receptors, indicating that this neurological mechanism is involved in disease severity and mortality [35]. It was recently demonstrated in hypertensive mice that in relation to the vitamin D/RAS interaction, ACE2/Ang (1-7)/MasR signaling cascade plays a role in vitamin D neuroprotective effects in the brain [36]; it was also observed that vitamin D leads a role of cofactor D in mitigating incident atrial fibrillation by inhibiting RAS [37]. Moreover, the re-adjustment of normal plasma levels of vitamin D [the major circulating form of vitamin D is calcidiol, 25-hydroxycholecalciferol, or 25-hydroxyvitamin D (abbreviated 25OHD)] in patients with vitamin D deficiency elicits peripheral RAS blockade [38]. In the hepatic cells, the exacerbated activation of RAS induces liver dysfunction and

CHAPTER 4

Anti-infectives to Combat Leishmaniasis

Shalini Roy[1], Anindita Ukil[1] and **Pijush K. Das[2,*]**

[1] *Department of Biochemistry, University of Calcutta, Kolkata 700019, India*

[2] *Infectious Diseases and Immunology Division, CSIR- Indian Institute of Chemical Biology, Kolkata 700032, India*

Abstract: Anti-infectives, by definition, refers to the drugs that can act against infection either by inhibiting the spread of infectious agents or killing them outright. These include a plethora of compounds that encompass antibiotics, anti-fungals, anti-helmintics, anti-malarials, anti-leishmanials, anti-protozoals, anti-tuberculosis, and antivirals properties. This chapter is dedicated to specifically focus on articles related to the anti-leishmanial therapeutics which entails drug development, techniques to improvise drug delivery, and identification of new cost-effective better therapeutics which would have immense potential to overcome all the limitations of the present-day therapies. Leishmaniasis is a dreaded parasitic disease caused by the protozoan parasites of the genus *Leishmania.* It can be categorized into three types: cutaneous, mucocutaneous, and visceral, amongst which visceral leishmaniasis (VL) is a neglected tropical disease that results in significant morbidity and mortality worldwide. Approximately 500,000 new cases per year of visceral leishmaniasis (VL) are supposed to occur globally, of which 90% of the new cases are found to affect just five countries, including India. Therapeutic measures those in vogue, again pose a number of serious limitations related to toxicity, lengthy regime, drug side effects, drug resistance, and cost, making overall treatment a complex issue. Again, VL poses unique problems in different settings which is a major threat for the choice of treatment. Furthermore, fundamental differences in the behavior of the causative parasites and the response of the host to the pathogen in different settings may also vary which can complicate the choice of treatment. The healthcare provision for VL patients in India is not up to the mark as it is a poorly standardized system of private care, which is associated with relatively high costs, thus making treatment difficult for the poor population. This is especially a ‘disease of the poor' mostly affecting the lower socio-economic population who is malnourished and cannot strictly abide by regular monitoring or follow-ups during treatment owing to poverty reasons. Drug resistance has become a growing limitation because the partially recovered patients has the possibility of developing parasites resistant to treatment due to insufficient drug dosage, which in turn gets transmitted to new patients causing primary drug resistance. To alleviate all these problems of current therapies encountered so far, it is a necessity of paramount importance to explore and develop new drugs that can enrich the known small armamentarium of anti-leishmanials. Discovering new chemotherapeutics that are

* **Corresponding author Pijush K. Das:** Infectious Diseases and Immunology Division, CSIR- Indian Institute of Chemical Biology, Kolkata 700032, India; Tel: 91-33-24140921;
E-mail: pijushdas@iicb.res.in

Atta-ur-Rahman (Ed.)

cheap, effective, less toxic and capable to overcome drug resistance will help to better combat the disease. Available treatment options are quite limited, and planning to switch to combination therapy is also equally important, as it can scale up treatment efficacy. Overall, this chapter aims to highlight the challenges of the current anti-leishmanial therapies, coupled with the unraveling of the new therapeutic modalities and their mechanisms of action which potentiates them as better anti-leishmanial agents, thereby overcoming the problems of present-day therapeutics. Furthermore, it will also shed light on the importance of various immunomodulators and investigational drugs which might come up as effective, remedial therapeutics against leishmaniasis, in the future trials.

Keywords: Amphotericin, Anti-Leishmanial, Antimonials, Chemotherapy, Cutaneous Leishmaniasis (CL), Drug Delivery, Drug-Resistance, Immunomodulators, Miltefosine, Visceral Leishmaniasis(VL).

INTRODUCTION

This chapter is dedicated to focus is fine on the anti-infectives that can be characterized as anti-leishmanial by virtue of their potent leishmanicidal effects. The chapter provides a comprehensive understanding of the past and present anti-leishmanials being used, their growing limitations, and it also sheds light on elucidation of novel therapeutics that might have the potential to circumvent all the present-day challenges.

Leishmaniasis

Leishmaniasis, caused by the protozoan parasite *Leishmania,* is a group of diseases involving self-healing cutaneous to fatal visceral leishmaniasis. They are of three types, cutaneous, mucocutaneous, and visceral. Cutaneous leishmaniasis (CL) is characterized by skin lesions on the exposed parts of the body. Mucocutaneous leishmaniasis (MCL) causes the destruction of mucous membranes of the nose, mouth and throat. Both CL and MCL are not fatal and sometimes self-healing also. However, visceral leishmaniasis (VL) is the most dangerous form and is fatal if not diagnosed early and treated properly. VL particularly affects the organs in the viscera like spleen, liver and bone marrow. It is prevalent in tropical and subtropical countries, including India. As far as death from protozoal infections are concerned, this disease stands second only to malaria [1]. It is considered as neglected tropical disease.

Causative Parasite and the Vector

It is really surprising that although all three types of the disease are caused by morphologically very similar parasites of the genus *Leishmania,* they elicit such a strikingly different pathological response. Thus, CL is caused by *L. major* and *L. tropica,* MCL is caused by *L. braziliensis* and *L. mexicana* and VL is caused by *L.*

donovani. Infected dogs, human beings, or wild canids may act as reservoirs for transmission of the parasite through the bite of the insect vector *Phlebotomus argentipes, P. papatasi,* and *P. sergentis* commonly known as sandfly. *Leish mania* parasites exist in two forms, promastigote and amastigote. Promastigote is long (10-20 µm), tubular-shaped with a long flagellum, and resides in the gut of the sandfly vector. Amastigote is much smaller, ovoid-shaped (1-2 µm in diameter), contains no flagella, and resides in the macrophages of mammalian host.

Life Cycle

Leishmania parasites have a digenetic life cycle consisting of a mammalian host and an insect vector, sandfly. As described earlier, transmission of the parasite between mammalian hosts occurs by the bite of the sandfly vector. The digenetic life cycle involves three developmental stages, namely, procyclic promastigotes, metacyclic promastigotes, and amastigotes (Fig. **1**) [2]. Amastigote is the infective form that replicates within host macrophages for its propagation.

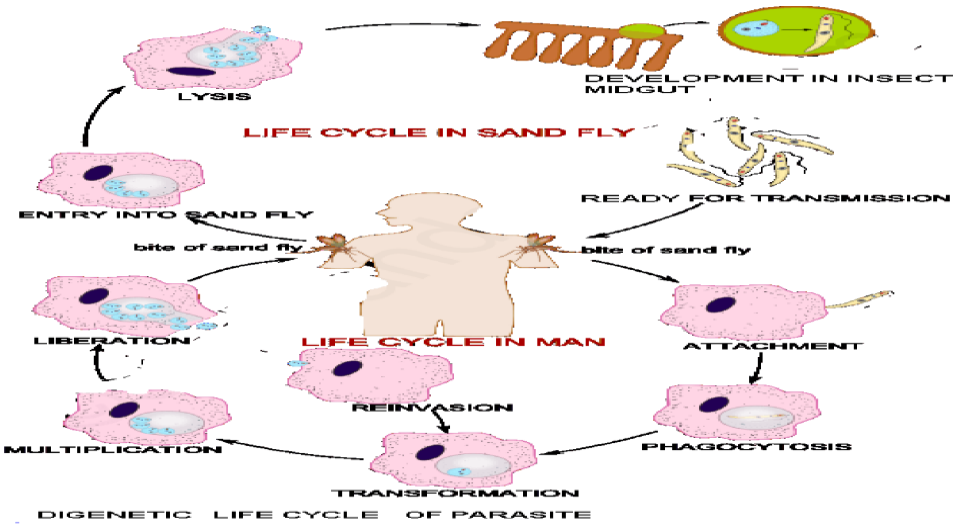


Fig. (1). Lifecycle of *Leishmania donovani* between two hosts, insect vector sandfly and human, is depicted above.

Mode of Transmission

Promastigote form in the sandfly gut subsequently transforms into the infective

CHAPTER 5

Anthelmintic Drug Discovery: Current Situation and Future Perspectives

María Gabriela Blanco[1,2], María José De Rosa[1,2,*] and **Diego Rayes[1,2,*]**

[1] *Laboratorio de Neurobiología de Invertebrados. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) CCT UNS-CONICET. Bahía Blanca, Argentina. Camino La Carrindanga Km 7, Bahía Blanca, Argentina*

[2] *Dpto de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. Bahía Blanca, Argentina. San Juan 670, Bahía Blanca, Argentina*

Abstract: Nematode parasites cause several neglected tropical diseases in humans such as lymphatic filariasis, onchocerciasis (river blindness), and soil-transmitted helminthiasis. Approximately 30% of the human world population is infected with at least one parasite and this prevalence could be even higher in rural areas and low-income countries. Although nematode infections are rarely lethal, they are associated with morbidity and severe consequences, particularly in children.

There are several concerns about the management and treatment of these diseases. Currently, the repertoire of nematocidal agents is limited, and these drugs are not 100% effective against all nematode parasitosis. In addition, the extensive use of these few drugs in massive administration campaigns in humans would probably lead to the development of resistance very soon. Further worsening the situation, the interest of the pharmacological industry in developing novel anthelmintics is low since these infections are mostly endemic in poor countries that do not constitute a profitable market. Under this alarming scenario, there is an urgent need to develop new and broad-spectrum antiparasitic drugs.

Traditional preclinical drug discovery is a long, expensive, and complex process. Thus, innovative strategies and alternative models, such as the free-living nematode *Caenorhabditis elegans*, are required to reduce costs and accelerate times. Its genetic amenability and the feasibility of performing high-throughput screening assays, convert this nematode into an excellent platform for nematocidal drug screening.

This chapter summarizes the current situation on antiparasitic drug discovery and discusses the use of *C. elegans* at the initial steps of drug development to accelerate the appearance of new drugs.

[*] **Corresponding author María José De Rosa and Diego Rayes:** Laboratorio de Neurobiología de Invertebrados. Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) CCT UNS-CONICET. Bahía Blanca, Argentina. Camino La Carrindanga Km 7, Bahía Blanca, Argentina; Tel: +54-0291-4861201; Fax: +54-0291-4861200; E-mail: mjderosa@criba.edu.ar, and Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB) CCT UNS-CONICET. Bahía Blanca, Argentina; Tel: +54-029--4861201; Fax: +54-0291-4861200; E-mail: drayes@criba.edu.ar

Atta-ur-Rahman (Ed.)

Keywords: Anthelmintic Drugs, *C.elegans*, Drug Development, Intestinal Infections, Neglected Diseases, Nematode Infections, Parasites, Tissue Infections.

INTRODUCTION TO PARASITIC NEMATODES

Parasitism is a form of ecological interaction, in which one member, the parasite, benefits from the use of resources gathered by another member, the host. In animals, the mechanisms for obtaining food have led to the development of predatory and parasitic ways of life. The predator feeds on another organism, the prey, usually hunted by himself and eventually killed. The parasite lives in biological association with another living being, the host, to ensure their own survival and reproduction. Generally, the predator is larger and more powerful than the prey; in contrast, the parasite is smaller and weaker than its host. Usually, parasites do not kill their hosts, but they can cause pathogenic actions by altering the homeostatic balance or the immune response.

Parasites are a heterogenous group of organisms divided into three main classes: protozoa (unicellular organisms that live inside or outside host cells), helminths (multicellular organisms that due to their size are solely extracellular pathogens) and ectoparasites (arthropods that live on the surface of the host´s body). Helminths (from the Greek *helmins*, meaning worms) include two major phyla: nematodes (roundworms) and platyhelminths (flatworms that comprise cestodes (tapeworms) and trematodes (flukes)). Several bacterias, rickettsias, viruses and fungi can also be considered parasites. However, to facilitate systematization, the study of these biological agents has been separated into different disciplines: bacteriology (bacterias, rickettsias and spirochetes), mycology (fungi), and virology (viruses). Therefore, the term parasites generally refers to protozoa and metazoans (helminths and arthropods) that benefit at the expense of other organisms (the host).

Parasites infect a wide range of species, including humans, companion animals, livestock, and crops producing a devastating impact on human life quality and economy. Parasitic diseases are a health problem all over the world. Most parasitic diseases are endemic in under-developed countries, due to inappropriate sanitary and infrastructure conditions associated with poverty. However, tourism and migratory movements have spread some parasitic infections to non-endemic regions as well.

Helminths: Phylum Nematoda

Nematodes (from the Greek *nemat* “threads” and *odes* “like”) or roundworms include around 25,000 species and constitute the third phylum of the Kingdom Animalia (behind the phylum Arthropoda and the phylum Mollusca) in number of

species, breadth of distribution, and amount of biomass. This group includes free-living non-parasitic members and parasitic species that infect plants, animals and humans causing important diseases such as filariasis, ascariasis, triquinellosis among others.

Nematodes are cylindrical worms, non-segmented, with tapering anterior and posterior ends and covered with a usually translucent, flexible acellular cuticle secreted by the hypodermis. Most of them are microscopic, such as *Trichinella spp.,* which is only a few mm long. However, some species such as *Ascaris spp.* can reach more than 30 cm long. They have simple anatomy, which includes digestive, reproductive, nervous and excretory systems. In contrast, nematodes do not have circulatory and respiratory systems. The digestive tract includes the foregut (stomodeum; buccal cavity and the muscular pharynx that pumps food through the gut), the midgut (intestine), and the hindgut (proctodeum; rectum and anus). Depending on their feeding habits, nematodes can contain specialized structures in their mouth. For instance, the parasitic nematode *Ancylostoma duodenale* contains tooth-like structures used to attach to the intestinal villi of the host [1]. Nematodes only have longitudinal body wall muscles. Synchronized dorsal and ventral contraction of these longitudinal muscles permits the typical back and forth thrashing movement of these worms. The nervous system consists of the nerve ring, a circumpharyngeal neuropil composed of neuronal processes whose cell bodies cluster in the head, and four longitudinal nerve cords that run along the length of the body in ventral, dorsal, and lateral positions.

Nematodes have well-developed reproductive systems adapted to produce large amounts of eggs. Most of them are sexually dimorphic, with females and males. However, some species can be hermaphrodites (*e.g*., *C. elegans*) or partheno genetic (parasitic females of *Strongyloides stercoralis*). The females lay eggs, usually after fertilization by males (in hermaphrodite individuals could also be by self-fertilization). Nematode eggs hatch into larvae (juvenile stages). Each larval stage finishes by a molt. All nematodes have four larval stages, with the first molt usually occurring in the egg. After the final molt, the nematodes differentiate into reproductive stages (hermaphrodites, males and females). Complete life cycles (from egg to egg) are generally short, ranging from four days (for the free-living *C. elegans*) to four-six weeks (for most parasite worms). When exposed to adverse conditions, several nematodes have the capacity of arresting their larval development. These arrested stages are generally non-feeding and can subsist on internal food stores for months until reencountering favorable conditions.

Overview of Nematode Infections

All species of vertebrates serve as hosts for parasitic nematodes. Some of these

CHAPTER 6

Therapeutic Targets for Emerging Zika Virus Infection and Vaccines in Clinical Trials

Deepthi Ramesh[1], Balaji Gowrivel Vijayakumar[1] and **Tharanikkarasu Kannan[1,*]**

[1] *Department of Chemistry, Pondicherry University, Kalapet, Puducherry, 605014, India*

Abstract: Zika virus is a mosquito-borne disease initially limited to sporadic cases in Africa and Asia. With the recent emergence of the Zika virus in Brazil in 2015, the virus rapidly spread throughout America. Even though most of the Zika virus infections have a mild influenza-like illness, severe manifestations were also observed, including Guillain-Barre syndrome in adults and microcephaly in babies born to infected mothers. Due to the severity of this disease, structural virologists quickly studied its different features. But, even with the elucidation of the viral genome, an effective treatment or suitable vaccine is not available for this disease so far. The viral vectors, pathogenesis, genetic diversity, and co-infection with other diseases remain unanswered. The production of an effective vaccine is hence a global health concern. This chapter discusses the emergence of the Zika virus and its detailed genome structure and replication cycle. The molecular pathogenesis and Zika viral therapeutics with detailed descriptions about the host and viral targets, investigational drugs, and vaccine candidates are explained here.

Keywords: Drug Discovery, Genome Structure, Host Protein Targets, Molecular Pathogenesis, Protease, RNA Polymerase, Vaccine, Viral Protein Targets, Zika Virus, Zika Virus Diagnostics, Zika virus Life Cycle.

ZIKA VIRUS DISCOVERY AND EPIDEMIOLOGY

Zika virus (ZIKV) is a positive single-stranded enveloped RNA virus in *the Flaviviridae* family and *flavivirus* genus. Dengue virus (DENV), Usutu virus (USUV), West Nile virus (WNV), Japanese encephalitis virus (JEV), Yellow Fever virus (YFV), and tick-borne encephalitis are other viruses that come under the flavivirus genus. Flaviviruses infect people from all around the world; 400 million people get infected per annum due to these viruses. ZIKV is transferred to humans through the infected bite of *Aedes* species mosquitoes, causing Zika fever with rashes, arthralgia, and conjunctivitis. Zika fever is often undetected or mis-

* **Corresponding author Tharanikkarasu Kannan:** Department of Chemistry, Pondicherry University, Kalapet, Puducherry, 605014, India; Tel:+91-413-2654411; Fax:+91-413-265 6740; E-mail:tharani.che@pondiuni.edu.in

Atta-ur-Rahman (Ed.)

taken as its symptoms are similar to other diseases like dengue fever or chikungunya [1]. Even after 60 years of its discovery in 1947, Zika fever was confined within African and Asian regions and was not regarded as a severe health threat until the early 21st century. However, in 2007, the ZIKV emerged again, leading to febrile illness in the Yap Islands of Federated States of Micronesia, and started a new chapter of ZIKV infections. Continuous ZIKV transmission in the Pacific islands (2014) and Brazil (2015) cemented that the virus had spread throughout the continent [1, 2].

In 1947, Kitchen, Dick, and Haddow initially identified the ZIKV from the serum of a sentinel monkey, Rhesus 766, from the Zika forest in Uganda, East Africa. The same team at the Virus Research Institute, Entebbe, Uganda, isolated the ZIKV from *Aedes africanus*, the vector for yellow fever virus, in 1948, providing the first evidence of human infection and confirming the *Aedes* species mosquitoes as the ZIKV vectors [3]. The clinical symptoms of ZIKV infection remained mysterious till scientist William Bearcroft auto inoculated with ZIKV and showed moderate symptoms like headache, fever, and nausea [4]. Amid a suspected yellow fever eruption in 1952, the first ZIKV human serum was isolated from a Nigerian girl. Also, two other patients showed a rise in serum antibodies against ZIKV [5]. In 1966, ZIKV was isolated from *Aedes aegypti* [6], and in 1977, Indonesia recorded the first human ZIKV infection in Asia [7]. However, for decades, ZIKV was considered as a mysterious virus with an unclear impact on the human population. In 2007, the first human transmission of ZIKV was identified outside of Asia and Africa. The Zika upsurge in an isolated island of Yap, Western Pacific, had 49 confirmed cases and 59 suspected infection cases with an additional 73% population having antibodies [8]. In 2008, two USA scientists who returned from southeast Senegal showed ZIKV infection-like symptoms, such as rashes, fatigue, headache, arthralgia, and one person was affected with haematospermia. One patient's wife became sick with no travel history but with similar symptoms. As ZIKV were identified in the semen samples, ZIKV transmission through sexual contact or body fluids was established [9]. In 2010, specimens from a child in Cambodia showed ZIKV infection, and in 2012, blood samples of a boy from the Philippines also tested positive for ZIKV infection [9]. During 2013 - 14, the eruption of ZIKV infection in French Polynesia was reported, with roughly 300 confirmed and over 19,000 suspected cases with Guillain-Barré syndrome (GBS) [10]. In Indonesia, during a dengue virus upsurge in 2014, one positive case of ZIKV infection was also detected, and from the infected person, many travelers from Thailand who visited Indonesia acquired ZIKV infections [9]. In 2014, New Caledonia reported an outbreak, and ZIKV sequence analysis marked two significant lineages, African and Asian. In 2015, 24 patients showed illness with rash, arthralgia, and conjunctivitis in Camaçari, Brazil. The identification of ZIKV RNA in seven

patients' serum confirmed the escalation of ZIKV in continental South America. Three patients had chikungunya RNA in serum, highlighting that Zika fever cannot be diagnosed with only clinical characteristics [8]. After the first reported cases, by 2016, Zika became an epidemic in Brazil, with microcephaly identified in children born to infected women. Phylogenetic analysis of ZIKV sequences from Brazil exposed the Asian lineage of the virus, which could have been introduced from the Pacific Islands in the 2014 FIFA World Cup or IVF World Sprint Championship. ZIKV was named a public health emergency of international concern (PHEIC) by WHO in February 2016 and later this status was withdrawn in November 2016. Singapore and Vietnam reported two outbreaks in Asia, and Thailand reported boundless transmission [11]. With 48 countries in the Western Hemisphere infected with ZIKV transmission by 2017, ZIKV became endemic in American regions, Southeast Asia, Central Africa, and the South Pacific [10]. Till now, 86 countries and territories have recorded ZIKV infection [12]. The significant events and timeline of ZIKV infections are given in Fig. **(1).**

Fig. (1). Significant events in Zika virus infection.

One factor involved in the rise of ZIKV strain in recent years with increased transmission potential and virulence can be attributed to the virus's genetic mutations because of the error-prone nature of viral polymerase. The factors like universal, demographic, technological, and social tendencies of population

CHAPTER 7

Agro-Industrial Waste: New Source of Raw Material for the Control of American Foulbrood in Honey Bees

Pablo Giménez-Martínez[1,2], Cristina Ramírez[3], Azucena Iglesias[1,2], Florencia Fangio[4], Sandra Churio[4], Matias Maggi[1,2] and **Sandra Fuselli[1,5]**

[1] *Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP, Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina*

[2] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*

[3] *Departamento de Química y Bioquímica, FCEyN, Universidad Nacional de Mar del Plata, Argentina*

[4] *Departamento de Química, Facultad de Ciencias Exactas y Naturales, Instituto de Investigaciones Físicas de Mar del Plata (IFIMAR), CONICET, Universidad Nacional de Mar del Plata, Mar del Plata, Buenos Aires, Argentina*

[5] *Comisión de Investigaciones Científicas (CIC), Buenos Aires, Argentina*

Abstract: Agro-industrial waste production represents an environmental problem, and its processing results in the obtainment of by-products that are rich sources of bioactive compounds. *Apis mellifera* are social insects that create the ideal conditions for the transmission of pathogens. American foulbrood (AFB) is the main pathology that affects bee brood; the causative agent is the Gram-positive bacteria *Paenibacillus larvae*. During the last years, there have been significant losses of hives by the use of synthetic antimicrobials for the control of AFB. The presence of antibiotics in honey and other products of the hive have generated concern in consumers regarding the risks of toxicity, negatively influencing the time of marketing and export. On the other hand, there is the possibility of the appearance of resistance by pathogenic microorganisms caused by incomplete treatments or an overdose of the antibiotic used. More than 70 plant extracts have been evaluated *in vitro* and *in vivo* against *P. larvae*; for this reason, the revaluation of biological waste material from agroindustry is being studied through pharmacodynamic and pharmacognosy analysis both in *P. larvae* and in the different stages of development of *A. mellifera*.

Therefore, this chapter proposes to investigate: (1) the effect of the application of fertilizer treatment on the hop cultivar in the composition and amount of secondary

* **Corresponding author Sandra Fuselli:** Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET, UNMdP. Centro de Asociación Simple CIC PBA, Mar del Plata, Argentina and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; Tel:(0054223) 4268051; E-mail: beedarian@gmail.com

Atta-ur-Rahman (Ed.)

metabolites, (2) the bioactivity of extracts of hop leaves against *P. larvae*, and *in vitro* toxicity in larvae and adult bees, and the effect on genes of the immune system of bees.

Keywords: Agro-Industrial Waste, American Foulbrood, Antimicrobial Activity, *Apis Mellifera*, Flavonoids, Honey Bee, *Humulus lupulus*, *Paenibacillus Larvae*, Phenolic Compounds, Saponins, Secondary Metabolites.

INTRODUCTION

Bees of the species *Apis mellifera* L. (Order Hymenoptera) are social insects that make up colonies of more than 50.000 individuals, generating the ideal conditions for the transmission of pathogens [1]. The most important diseases that affect bees are American foulbrood (AFB), European foulbrood, Nosemosis and Varroosis, which can affect adult bees, larvae or both stages [2, 3]. *Apis mellifera* is responsible for pollinating 77% of the plant species responsible for the production of food resources that sustain the world population; due to this, they play an important role on society not only for the commercial products of the hive but also for contributing to the balance and proper functioning of ecosystems, and consequently to biodiversity. They are insects that provide significant environmental services due to their performance as biological indicators and pollination [4]. The current beekeeping production is negatively influenced by the diseases that affect the colonies that generate high mortality, and therefore, decrease the productive yield.

American Foulbrood

Characteristics of the Disease

This disease negatively affects *A. mellifera* during the larval stage; adult bees are asymptomatic passive or active carriers, being able to remain latent and appear suddenly due to stress factors, external conditions, genetic resistance or unknown causes [5]. It is recognized as the most destructive and widespread of all bacterial diseases of bee brood that not only kills the larvae but is potentially lethal to infected colonies. This disease is within the OIE Terrestrial Animal Health Code, and member countries are required to declare it.

Paenibacillus Larvae, the Causal Agent

The disease is generated by Gram-positive bacteria, rod-shaped (2.3 to 5 μm long by 0.5 to 0.7 μm wide), grouped in pairs, alone or in short chains, measuring 1.3 μm x 0.6 μm and mobile with flagella. Its main characteristic is the possibility of forming spores, which can be ellipsoidal, central or terminal, with high tolerance to high temperatures, and resistance to the action of chemical disinfectants, such

as chlorine, iodine-based products and ultraviolet radiation. The spores can remain infective for more than 40 years, being responsible for the beginning of the disease cycle [6 - 8] and only infect larvae of the genus *Apis* [9].

PCR analysis showed four genotypes of *P. larvae* ERIC (I-IV), showing that the resulting classification correlates with phenotypic differences [7]; the latest studies by Beims, Bunk [10] revealed a new genotype, *P. larvae* ERIC V.

Pathogenesis and Symptoms

The spores enter the interior of the hive through foraging bees, bees from infected hives, contaminated beekeeper tools, by the introduction of pictures with infected offspring, through feeding with contaminated honey and any exchange of material from diseased hives. Once inside the hive, the spores are brought to the brood by the nurse bees along with the food. The larvae ingest these spores that adopt their vegetative forms, given the appropriate conditions they have in the intestine, such as pH and percentage of oxygen. When the larva reaches its pre-pupal stage, the bacteria that have not yet been eliminated in the feces migrate, entering the endothelial cells of the intestine thanks to their flagella, reach the hemolymph and reproduce until they cause death due to generalized septicemia [2, 6, 11]. After several days, the pupa dries up and gradually acquires an increasingly darker coloration due to the pigmentation of *P. larvae*, being called at this stage as "scale" having a very high infective power, and may contain up to 2.5 billion spores, being a very important source of their dissemination [12].

Disease Control

Due to the characteristics of the disease, once AFB is detected in a region, it is very unlikely that it can be eradicated. Any of the methods mentioned below must inevitably be complemented with an intensive program of periodic inspections of the apiaries, within a maximum interval of 90 days, including the winter season. It is important to consider that if a single colony is left in the field untreated, it can destroy the work of several years of control.

Conventional Chemotherapy Treatment

This disease causes severe economic damage to the beekeeping sector in many honey-producing countries, because the presence of antibiotics in honey and other beehive products has generated concern in consumers regarding the risks of toxicity, negatively influencing the moment of its commercialization and export [8]. Likewise, there is the possibility of the appearance of resistance by pathogenic microorganisms, caused by incomplete treatments or an overdose of the antibiotic used.

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

J

L

M

N

O

P

R

S

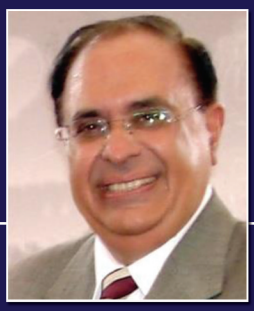
T

V

W

Y

Z



PROF. DR. ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.