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Therapeutic Replacements to Antibiotics

Mandeep Kaur , Satish Gupte, Tanveer Kaur

Department of Microbiology, Gian Sagar Medical College & Hospital, Rajpura, India.

Corresponding author:
Prof. (Dr.) Satish Gupte

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Abstract

For more than half a century, the human society has been relying primarily on antibiotics to treat infectious diseases caused by pathogenic bacteria. After the development of antibiotics, a general belief arose that the problem of bacterial infections would be solved. But the more use of antibiotics for the treatment of bacterial infection resulting in Multidrug-resistant bacterial infections. The emergence of infectious disease caused by drug-resistant bacteria requires alternatives to conventional antibiotics. Immunity play important role to treat infectious diseases. Certain type of immunomodulators are used to boost the immunity against infectious diseases. Immunomodulators are biological or synthetic substances that can modulate any aspect

of the immune system including both adaptive and innate arms of the immune system. Vaccines play an important role in providing and improving immunity to a particular disease. Monoclonal antibodies are currently being developed against certain infectious agents, including cytomegalovirus and human immunodeficiency virus. Prebiotics and probiotics are used to promote the growth and multiplication of specific beneficial gut microflora. Micronutrients such as selenium, zinc, and vitamin A and macronutrient such as Protein, Fat, Carbohydrates known to modulate immunity . Bacteriophages, Bacterial cell wall hydrolases and Antimicrobial peptides are also used as alternative to antibiotics in certain bacterial infections. Bacteriophages are ‘bacterium eaters’ that kill bacterium by causing its lysis (bacteriolysis). This review article highlights various types of alternatives to antibiotics which are used for the treatment of bacterial infections.

Keywords: Antibiotics, Immunity, Vaccine, Bacteriophages, Antimicrobial peptides.

Introduction

For more than half a century, the human society has been relying primarily on antibiotics to treat infectious diseases caused by pathogenic bacteria. However, the emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal agricultural usage has made antibiotics less and less effective [1]. After the development of antibiotics, a general belief arose that the problem of bacterial infections would be solved. Nonetheless, pathogens have evolved sophisticated mechanisms of drug resistance. Due to their high capacity to acquire resistance to antibiotics, there are not enough chemotherapeutics to destroy bacteria and to counteract the problem of infections in the human population [2]. Multidrug-resistant bacterial infections cause significant patient mortality and morbidity, and rising antibiotic resistance is a serious threat to the vast medical achievements made possible by antibiotics over the past 70 years [3]. Nowhere is the concept of antimicrobial resistance better portrayed than with the gram-negative bacilli, which have proven to be tough adversaries for clinicians and researchers alike [4]. However, an increased frequency of bacterial mutations has resulted in a significantly increased incidence

of antibiotic resistance. The horizontal spread of resistance genes to other bacteria of the same or different species has been shown to rapidly create bacterial populations with (a) an increased ability to degrade antibacterial compounds; (b) decreased permeability; (c) decreased affinity for the antibiotic; or, finally, (d) increased efflux of many different antibiotics [5] The emergence of infectious disease caused by drug-resistant bacteria requires alternatives to conventional antibiotics [6]. The search for new drugs is becoming critical because of the growing concern over the failing antibiotic drug discovery pipeline. There is a great deal of interest to investigate alternatives and natural antimicrobial agents for the treatment.

Therapeutic replacements to Antibiotics

Immunity

This may be defined as the body's ability to identify and resist large numbers of infectious and potentially harmful microorganisms, enabling the body to prevent or resist diseases and inhibit organ and tissue damage. The immune system is not confined to any one part of the body. Immune stem cells, formed in the bone marrow, may remain in the bone marrow until maturation or migrate to different body sites for maturation. Subsequently, most immune cells circulate throughout the body, exerting specific effects. The immune system has two distinct but overlapping mechanisms with which to fight invading organisms, the antibody-mediated defense system (humoral immunity) and the cell-mediated defense system (cellular immunity)[7].

Immune system

The basic architecture of the immune system is multilayered, with defenses on several levels. Most obvious and primary is the skin: the first barrier against infection. Another is physiological, where conditions like the temperature and pH of the body provide inappropriate living conditions for foreign organisms. Once pathogens have successfully entered the body, they are addressed by the innate and/or the acquired or adaptive immune

system. Both systems consist of a multitude of cells and molecules that interact in a complex manner to detect and eliminate pathogens. Detection and elimination depend upon chemical bonding: surfaces of immune system cells are covered with various receptors, some of which chemically bind to pathogens, while others bind to other immune system cells or molecules to enable the complex signaling system that mediates the immune response [8]. An infectious disease can occur only in a susceptible host, with susceptibility being a function of the effectiveness of the immune response. Damage to the host may be caused directly by microbial factors, can result from host factors—such as inflammatory responses—or both [9]. Some microbial diseases cause damage to the host because an overly vigorous immune response leads to excessive inflammation, others occur because immune responses are insufficient and require bolstering [10].

Immunity Boosters

Immunomodulators

These are biological or synthetic substances that can stimulate, suppress or modulate any aspect of the immune system including both adaptive and innate arms of the immune system. They are a diverse array of recombinant, synthetic and natural preparations, often cytokines. Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria are already licensed for use in patients. Others including IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG), oligodeoxynucleotides and glucans are currently being investigated extensively in clinical and preclinical studies. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases [11].

Classification of immunomodulators

Clinically, immunomodulators can be classified into the following three categories:

Immunoadjuvants are used to enhance the efficacy of vaccines and therefore could be considered specific immune stimulants. Immunoadjuvants hold the promise of being the true modulators of the immune response. It has been proposed that they be exploited as selectors between cellular and humoral helper T1 (Th1) and helper T2 cells (Th2), immunoprotective, immunodestructive, and reagenic [immunoglobulin E (IgE)] versus IgG type immune responses—posing a real challenge to vaccine designers [12].

Immunostimulants are inherently non-specific as they are envisaged as enhancements to a body's resistance to infection. They can act through innate as well as adaptive immune responses. In healthy individuals, the immunostimulants are expected to serve as prophylactic and promoter agents, i.e., as immunopotentiators, by enhancing the basic level of immune response. In the individual with impairment of immune response, they are expected to act as immunotherapeutic agents [13].

Immunosuppressants are a structurally and functionally heterogeneous group of drugs, which are often concomitantly administered in combination regimens to treat various types of organ transplant rejection and autoimmune diseases [14].

Vaccines

A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters. Vaccines can prevent or ameliorate morbidity from infection. The effectiveness of vaccination has been widely studied and verified; for example, the influenza vaccine,[15] the human papillomavirus

(HPV) vaccine,[16] and the chicken pox vaccine [17].The World Health Organization (WHO) reports licensed vaccines are currently available to prevent, or contribute to the prevention and control of, 25 vaccine-preventable infections [18].

Vaccine types

Live attenuated vaccine : Variolation, a procedure developed in China and India ~1000 AD used a live smallpox vaccine to generate immunity—employing several different techniques ‘well individuals’ were exposed to variolous material from a human with a milder form of smallpox—presumably in the expectation that this would cause less severe disease in the recipient—an early form of ‘attenuation’ [19,20].Live attenuated vaccines that might be used in the occupational setting include measles, mumps, rubella and chickenpox. Using measles as an example, the vaccine is injected deep sc/im where virions enter various cell types using receptor-mediated endocytosis. Within the cytosol, proteolytic degradation of viral proteins occurs, the peptides produced are then loaded onto major histocompatibility complex type I molecules and the complex is displayed on the cell surface. Circulating cytotoxic T cells (Tc) with the appropriate high-specificity TCRs are able to recognize the complex and release cytokines that instruct the (infected) cell to undergo programmed suicide (apoptosis) [21].

Killed/inactivated vaccines:

The term killed generally refers to bacterial vaccines, whereas inactivated relates to viral vaccines [19]. Typhoid was one of the first killed vaccines to be produced and was used among the British troops at the end of the 19th century. Polio and hepatitis A are currently the principal inactivated vaccines used in the UK—in many countries, whole cell pertussis vaccine continues to be the most widely used killed vaccine[20]. Hepatitis A is an example of an inactivated vaccine that might be used by occupational health practitioners. It is a formalin inactivated, cell culture adapted, strain of HAV; vaccination generates neutralizing antibodies and protective efficacy is in excess of 90%. Primary immunization with a booster between 6 and 12 months after the first should provide a minimum 25 years protection [19].

Subunit vaccines : Subunit vaccines are a development of the killed vaccine approach: however, instead of generating antibodies against all the antigens in the pathogen, a particular antigen (or antigens) is used such that when the antibody produced by a B cell binds to it, infection is prevented, the key therefore to an effective subunit vaccine is to identify that particular antigen or combination of antigens [19,20]. Hepatitis B and *Haemophilus influenzae* b (Hib) are examples of subunit vaccines that use only one antigen; influenza is an example of a subunit vaccine with two antigens (haemagglutinin and neuraminidase).

Toxoid vaccines : Certain pathogens cause disease by secreting an exotoxin: these include tetanus, diphtheria, botulism and cholera—in addition, some infections, for example pertussis, appear to be partly toxin mediated [19,20]. Tetanus toxoid vaccine is manufactured by growing a highly toxigenic strain of *Clostridium tetani* in a semi-synthetic medium: bacterial growth and subsequent lysis release the toxin into the supernatant and formaldehyde treatment converts the toxin to a toxoid by altering particular amino acids and inducing minor molecular conformational changes. Ultrafiltration then removes unnecessary proteins left as a residual from the manufacturing process to produce the final product. The toxoid is physico-chemically similar to the native toxin thus inducing cross-reacting antibodies but the changes induced by formaldehyde treatment render it non-toxigenic [22].

Monoclonal Antibodies

Antibodies are proteins produced by the B lymphocytes of the immune system in response to foreign proteins, called antigens. Antibodies function as markers, binding to the antigen so that the antigen molecules can be recognized and destroyed by phagocytes. The part of the antigen that the antibody binds to is called the epitope. The epitope is thus a short amino acid sequence that the antibody is able to recognize [23]. Monoclonal antibodies are currently being developed against certain infectious agents, including cytomegalovirus and human immunodeficiency virus. Palivizumab is a humanised IgG1 monoclonal antibody licensed for respiratory syncytial virus (RSV) prophylaxis. RSV is the most important

respiratory pathogen in infants and approximately 1% of infections require hospitalization [24]. Palivizumab binds to the F protein of RSV subtypes A and B. A single intravenous infusion of Palivizumab significantly reduces the RSV concentration in tracheal secretions for two days in children mechanically ventilated for RSV [25]. It has been demonstrated that combination therapy with mAb cocktails prevents escape variants for many viruses, including influenza [26], coronavirus [27]. However, the use of antibodies in the cocktail mode, as an approach to improve their effectiveness, is already recognized for other pathogens or toxins. In the case of tetanus toxin, it has been reported that combining the action of three out of four antibodies increased the neutralizing activity up to 200 times [28]. In the case of botulinum toxin, neutralizing activity has been reported up to 20,000 times higher when using a mixture of three monoclonal antibodies [29].

Polyclonal Antibodies (Antiserum)

Antibodies can be administered as human or animal plasma or serum, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, as high-titer human IVIG or IG from immunized or convalescing donors, and as monoclonal antibodies (MAb)[30]. Respiratory tract infections secondary to group A Streptococcus, Streptococcus pneumoniae, Haemophilus influenzae type b, and to a lesser extent Neisseria meningitidis are more frequent in patients with primary antibody deficiencies and that these infections can be markedly reduced by regular administration of immunoglobulin [31]. Further, specific animal antisera to these organisms were used in the early 1930s for treatment of severe infections (e.g., meningitis) [32]. Many of the adverse consequences of diphtheria result from the action of its potent toxin on the heart, central nervous system, and other organs[33]. The prompt use of equine diphtheria antitoxin is indicated in all infections, in addition to antibiotics [34]. Pertussis antiserum was used in the 1930s for treatment of pertussis [35]. Antibody for the prevention and treatment of tetanus dates from 1890, when serum prepared from immunized horses was used in the treatment of severe tetanus since toxin neutralization is a crucial part of the treatment [36]. Food-borne and wound botulism are treated with a

trivalent (types, A, B, and E) equine antitoxin available through the Centers for Disease Control and Prevention [37].

Prebiotics

Prebiotics are a group of bio-molecules grouped together by virtue of their capability to promote the growth and multiplication of specific beneficial gut microflora. Prebiotics may be defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon”. As it matches with certain aspects of dietary fiber, the updated version of prebiotics encompasses “selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health” [38]. The prebiotic targets a range of different physiological functions including better gut health, higher mineral absorption, lowering of cholesterol, immune stimulation and pathogen exclusion[39].

Probiotics

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”[40]. It is now well established that some of the infections and disorders in the human body, such as irritable bowel syndrome, inflammatory bowel disease, and antibiotic-induced diarrhea, could be due to deficient or compromised intestinal microflora, and probiotics have been considered to be one of the disease control strategies to overcome such disorders [41].

Micronutrients and Macronutrients

Micronutrients such as selenium, zinc, and vitamin A known to modulate immunity . Limited maternal dietary selenium also restricts transmission to the neonate that has been reported to result in impaired *in vitro* activation of thymocytes, and decreased proportions of circulating adaptive immune cells in the neonate [42]. Randomized controlled trials of zinc

supplementation in small for gestational age term infants have resulted in decreases in diarrhea, pneumonia infection, and may reduce overall mortality in some settings [43]. Vitamin A is crucial for integrity of barriers, lymphocyte proliferation, and cytotoxic T-cell activity, its deficiency reduces the number of circulating immune cells and complement proteins [44]. Human macronutrient (Protein, Fat, Carbohydrates) deficiencies primarily arises, when populations suffer acute or seasonal periods of starvation. These deficiencies disturb and alter the metabolic functions of the body. The immune system appears to be intrinsically tied to metabolic functions, and depending on timing of macronutrient deficiency, it may have long-term implications due to lasting epigenetic modifications [45].

Bacteriophages

Discovered independently by Frederick Twort and Félix d’Hérelle respectively in 1915 and 1917, phages are ‘bacterium eaters’ that kill bacterium by causing its lysis (bacteriolysis). Because of the actual rise of antibiotic-resistant bacteria, a revitalization of interests on phage therapy has been observed in the last two decades in western countries [46]. It is estimated that there are about 10^{31} phages on earth and approximately 5100 have been identified and reported towards the end of last century [47]. The most common mode of action of phages is the bacteriolysis, which occurs naturally at the end of the phage lytic cycle, by disruption of the cell wall caused by the virolysin-holin system or the single lytic factor. It could also happen in the adsorption stage if a high multiplication of infection (MOI) is used, in which a substantially large number of phage particles attach to the same bacterial cell [48]. Another mode of action involves genetically modified phages, especially filamentous ones, which do not cause cell lysis and cannot be used directly for phage therapy. Hagens and Blasi (2003) and Hagens *et al.* (2004) have shown that *Pseudomonas aeruginosa* filamentous phage can be genetically modified by replacing the transportation gene (i.e. the gene involved in the extrusion of phage particles from the host bacterium) with a restriction enzyme gene so that the phages loses the ability to extrude from bacterial cells for its multiplication, but acquires the ability to digest the bacterial nucleic acid [49].

There is an accumulation of evidences that phages can be employed for clinical treatment or prevention of infectious diseases caused by both gram positive (G^+) and gram-negative (G^-) bacteria [50]. After the works of Smith *et al.* (1987) on the treatment of *Escherichia coli* infections using experimental mice and calves, there have been many published reports on the successful efficacy of phages against experimental infections by G^- and G^+ bacteria, mostly in animal models [51]. Moreover, phages were also shown to be effective for the elimination of food poisoning pathogens such as *Listeria monocytogenes*, *Campylobacter jejuni*, and *Salmonella* spp. [52]. On 18 August 2006, Food and Drug Administration (FDA) approved the use of phages for the treatment of ready-to-eat meat: a combination of six viruses was designed to be sprayed on ready-to-eat meat to eradicate strains of *L. monocytogenes* [53]. There is no doubt that phages, after extensive studies and careful selection of phage candidates, will eventually become one of the most effective antibacterial alternatives.

Bacterial cell wall hydrolases

Bacterial cell wall hydrolases (BCWH) are enzymes that degrade peptidoglycan, the major component of the bacterial cell wall, and cause bacteriolysis. Different forms of BCWH can be used to treat infectious diseases, including purified native enzymes, denatured enzymes, partial digests [54], and recombinant proteins endogenously over-expressed in transgenic animals or plants for enhancement of host defense. BCWH classify according to their sources into the following categories: (i) lysozymes, BCWH of eukaryotic origin and components of the innate defence system of their producers; (ii) autolysins, bacteria encoded by BCWH that have a variety of physiological functions in the bacterial life cycle; and (iii) virolysins, phage-encoded BCWH responsible for the lysis of bacterial cells and the release of phage particles at the end of a phage life cycle. The primary bactericidal mechanism of BCWH is the lytic enzymatic activities of these enzymes, i.e. attacking specific sites in the peptidoglycan network, leading to peptidoglycan hydrolysis and consequently bacteriolysis[54]. A secondary bactericidal mechanism of BCWH, the nonlytic mechanism,

has also attracted the attention of the research community in the recent years [55]. The nonlytic mechanism is based on the cationic and amphiphilic properties of BCWH or its derived peptide to provoke membrane perturbation or to activate the autolytic system of bacteria. Extensive studies on the antimicrobial efficacy of different groups of BCWH, mostly lysozymes [56] and virolysins have been carried out in the last two decades. Lysozymes were shown to have antimicrobial activities towards bacteria, fungi, and viruses [57]. They are mainly used in food preservation and processing, but also have applications in veterinary and human medicine. On the other hand, virolysins have the capacity of rapidly killing pathogenic G⁺ bacteria on a generally species-specific basis, *in vitro* or *in vivo*. Since 2000, some virolysins have been demonstrated to be efficient and safe antimicrobials, and could potentially be used for the control of pathogens on mucous membranes or as biowarfare counter measures for *Bacillus anthracis* [58]. Interestingly, virolysins are rapidly effective at low dosages in the order of milligrams or even micrograms per litre [58].

Although autolysins are in general less studied as alternative antimicrobials, the concept of activating the autolytic system of pathogenic bacteria using cationic peptides and other compounds has been investigated with encouraging results [59]. However, no reports have been found regarding the clinical use of exogenous autolysins for the treatment of infectious diseases, except the suggestion that they might be used as vaccines because of their ability to provoke immunological responses against their corresponding bacteria [60].

Antimicrobial peptides

Antimicrobial peptides (AMP) are another major group of promising novel alternatives to antibiotics based on their effectiveness, safety, and enormous diversity. This is a large family of naturally occurring peptides from diverse sources, having diverse structures and functionalities. AMP according to their origins are Eukaryotic AMP, Bacteriocins, and Phage-encoded AMP. AMP have a large variety of different bactericidal mechanisms.

Although many of them still need to be elucidated, four of them are well understood and presented here. The first mechanism, thought to be the killing mechanism of the majority of eukaryotic AMP, is the formation of ion channels or pores across the cytoplasmic membrane of bacteria, which causes membrane perturbation, dissipation of the electrochemical gradient across the cell membrane, and loss of cell content [61]. The second mechanism is the inhibition of cell wall biosynthesis. For example, several short members of the lipid II-targeting antibiotics use an alternative bacteriolytic mechanism by removing the lipid II from the cell division site (or septum) to block cell wall synthesis [62]. The third mechanism will kill bacteria via the ribonuclease (RNase) or deoxyribonuclease (DNase) activities of some AMP. For example, colicin E9 kills sensitive *E. coli* cells by DNA degradation (i.e. the DNase activity) [63] and colicin E3 by catalysing a specific ribonucleolytic cleavage of 16S rRNA (i.e. the RNase activity) [64]. The fourth mechanism is used by phage tail-like bacteriocins to kill bacteria through specific binding of bacteriocins to the bacterial receptor, which provokes depolarization and perforation of the cytoplasmic membrane, inducing membrane perturbations [65]. Extensive studies have been carried out on the antimicrobial efficacy of different families of AMP. AMP of prokaryotic origins, such as bacteriocins, have been demonstrated to have high efficacy in eliminating bacterial species closely related to the producing strains [66]. For instance, local injections of staphylococcin A-1262a was used to treat 50 patients with a variety of staphylococcal lesions and a complete recovery was observed in 42 of them [67]. A comprehensive review on the antimicrobial efficacy of AMP has been published recently [68], which summarizes the potent activities of AMP against a broad range of micro-organisms encompassing G⁻ and G⁺ bacteria, fungus, parasites, and enveloped viruses, as well as the importance of AMP in inflammatory conditions including psoriasis, respiratory disorder, inflammatory lung disease, inflammatory bowel disease (IBD), rheumatoid arthritis, and atherosclerosis.

Conclusion

Immunomodulatory regimens offer an attractive approach as they often have fewer

side effects than existing drugs, including less potential for creating resistance in microbial diseases. In the individual with impairment of immune response, immunostimulants are expected to act as immunotherapeutic agents. Monoclonal antibodies are currently being developed against certain infectious agents, including cytomegalovirus and human immunodeficiency virus. The prebiotics and probiotics target a range of different physiological functions including better gut health, immune stimulation and pathogen exclusion. Micronutrients such as selenium, zinc, and vitamin A modulate immunity. Bacteriophages can also act as alternatives to antibiotics as they cause direct bacteriolysis. Bacterial cell wall hydrolases (BCWH) are enzymes that attack specific sites in the peptidoglycan network, leading to peptidoglycan hydrolysis and consequently bacteriolysis. Antimicrobial peptides (AMP) inhibit cell wall biosynthesis and kill bacteria via the ribonuclease (RNase) or deoxyribonuclease (DNase) activities. So all these can act as good alternatives to antibiotics in tackling with pathogenic bacteria without the problem of resistance posed by bacteria.

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Authors Column

Satish Gupte MD is Professor and Head, Department of Microbiology, Gian Sagar Medical College and Hospital, Ramnagar, Rajpura, Punjab, India. He has been teaching microbiology to medical, dental, nursing, paramedical undergraduate and postgraduate students for more than 37 years. He has also worked as Professor and Head, Department of Microbiology, Government Medical College and Associated Hospitals, Jammu, Jammu and Kashmir, India. He has published over 50 research papers in national and international journals besides authoring over 14 books touching various aspects of medical microbiology and blood transfusion medicine. He has been Examiner of MSc, BDS, MBBS and MD (Microbiology) of many universities of India. He has to his credit *Man of the Year Award (2002)* by American Biographical Centre, New York and his biographical sketch appeared in *Who's Who World*, USA.

