A STUDY ON HEAMPHILUS INFLUANZAE TYPE B DISEASE CAUSING ANTIGENS: AN APPROACH OF EPITOPE PREDICTION, ANTIGENICITY AND IMMUNOGENICITY PREDICTION

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REVIEW ARTICLE

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ABSTRACT

Vaccines work by mimicking disease agents and stimulating the immune system which in turn builds a defence mechanism against the disease causing agents. Some of the vaccines contain a part of the disease causing agents which are either weakened or dead. Apart from using vaccines only for viral infections, utilizing the same against Cancers both as therapeutic and preventative has captured huge interest. The use of Cancer vaccines in cancer therapies is called immunotherapy which is done either by specific cancer vaccine or universal cancer vaccine which contain tumour antigens that stimulate the immune system which in turn initiate various mechanisms that terminate tumour cells and prevents recurrence of these tumours. Hemophilus influenzae is a disease causing virus & here we have done a brief study about the different antigens of the b strain of Haemophilus influenzae and compare them using various bioinformatics tools to get the most effective antigen of the above mentioned strain.

Keywords: Heamphilus Influanzae, Antigenicity, Immunogenicity, epitope prediction.

INTRODUCTION

Haemophilus influenzae is an important human-restricted Gram-negative bacterial pathogen, which can cause severe invasive disease, such as meningitis, sepsis, and bacteremic pneumonia in susceptible individuals. Some strains of H. influenzae have a polysaccharide capsule representing the major virulence factor and antigen of this bacterial species. On the basis of the antigenic properties, six serotypes of encapsulated H. influenzae are distinguished (a, b, c, d, e, and f), and there are also non-encapsulated or nontypeable H. influenzae (NTHi). Encapsulated strains exhibit a higher ability to cause invasive disease because the capsule prevents complement-mediated bacteriolysis in the absence of opsonizing antibody. (1,2) Normal individuals can carry H. influenzae in their naso- and oropharynx, and the carriage is considered as the major factor inducing the development of natural immunity against the pathogen, along with exposure to some cross-reactive environmental antigens. The invasive disease mostly affects young children (below 2 years of age), as well as the elderly and immunocompromised individuals. One particular serological variant, H. influenzae serotype b (Hib), was the major cause of bacterial meningitis in young children worldwide before the conjugate Hib vaccine became available in the late 1980s. Pediatric vaccination against Hib has resulted in a dramatic decrease in the incidence rates of invasive Hib disease in all countries where the vaccine has been included in the national immunization programs. However, Hib vaccination does not confer protection against other serotypes of H. influenzae. Until recently, the significance of other serotypes of H. influenzae in the etiology of invasive bacterial infections has been largely overshadowed by Hib. (3, 4) However, it is obvious that other serological types of H. influenzae besides Hib cause significant
morbidity and mortality; moreover, their prevalence appears to be increasing in the Hib vaccine era. During the last decade, an increase in the prevalence of infections caused by NTHi has been reported worldwide, suggesting strain replacement following elimination of Hib from populations with high Hib vaccine coverage, as a new ecological niche became available for colonization with non-Hib strains of H. influenzae. Although an alarming trend towards an increase in the incidence of severe disease caused by NTHi has been now recognized in many countries, less attention is paid to H. influenzae serotype a (Hia), which appears to be present in certain geographic regions and among specific populations only. As most of cases of Hia disease are sporadic, the published reports are not always consistent in their findings. While invasive Hia disease has suffered from inadequate surveillance worldwide, Hia is now recognized as an important pathogen causing serious disease comparable to Hib in severity and case-mortality rates. For example, the case-fatality rate of invasive Hia disease among pediatric cases reported by the Canadian Immunization Monitoring Program ACTive (IMPACT) centers in 1996–2001 reached 16%. Remarkably, the highest incidence rates of invasive Hia disease have been found in some indigenous populations, such as North American Indians and Inuit of Alaska and Northern Canada, reaching the order of magnitude of the incidence rates of Hib in the pre-Hib vaccine era. The reasons for an increased susceptibility to Hia infection among specific populations groups are unknown. The goal of this paper is to summarize the current knowledge on Hia global epidemiology and to discuss potential prevention of this infection using specific immunization. (5, 6)

Clinical features

Clinical categories of invasive disease caused by Hib include meningitis, epiglottitis and a range of other infections such as septic arthritis, cellulitis and pneumonia. Hib is rarely isolated from the blood without a focal infection such as the above being evident or developing subsequently. The classical clinical signs of meningitis – neck stiffness and photophobia – are often not detected in infants, who present with drowsiness, poor feeding and high fever. Epiglottitis (inflammation of the epiglottis) presents with respiratory obstruction, associated with soft stridor and often drooling in a pale, febrile, anxious child who remains upright to maximize his or her airway. Meningitis and epiglottitis are almost invariably fatal without appropriate treatment. The case-fatality rate for Hib meningitis in developed countries is at least 3% even with treatment and 15 to 30% of survivors have permanent neurological sequelae. There are no specific clinical features of any of the focal infections due to Hib that enable them to be differentiated from those due to other organisms. However, before the introduction of Hib vaccines, epiglottitis was due to Hib in over 95% of cases. (7-9) Non-typeable Haemophilus influenzae strains may occasionally cause invasive disease, but are a common cause of otitis media in children and bronchitis in adults. Hib vaccines are not effective in preventing NTHi infections.

Structure and growth factor

Haemophilus influenzae is a gram-negative coccobacillus. It is generally aerobic but can grow as a facultative anaerobe. In vitro growth requires accessory growth factors; including “X” factor (hemin) and “V” factor (nicotinamide adenine dinucleotide [NAD]). Chocolate agar media are used for isolation. H. influenzae will generally not grow on blood agar, which lacks NAD. The outermost structure of H. influenzae is composed of polyribosyl-ribitol-phosphate (PRP), a polysaccharide that is responsible for virulence and immunity. Six antigenically and biochemical distinct capsular polysaccharide serotypes have been described; these are designated types a through f. In the prevaccine era, type b organisms accounted for 95% of all strains that caused invasive disease.
Disease

Invasive disease caused by H. influenzae type b can affect many organ systems. The most common types of invasive disease are meningitis, epiglottitis, pneumonia, arthritis, and cellulitis. Meningitis is infection of the membranes covering the brain and is the most common clinical manifestation of invasive Hib disease, accounting for 50% – 65% of cases in the prevaccine era. Hallmarks of Hib meningitis are fever, decreased mental status, and stiff neck (these symptoms also occur with meningitis caused by other bacteria). Hearing impairment or other neurologic sequelae occur in 15% – 30% of survivors. The case-fatality rate is 2% – 5%, despite appropriate antimicrobial therapy. (10)

Epiglottitis is an infection and swelling of the epiglottis, the tissue in the throat that covers and protects the larynx during swallowing. Epiglottitis may cause life-threatening airway obstruction.

Septic arthritis (joint infection), cellulitis (rapidly progressing skin infection which usually involves face, head, or neck), and pneumonia (which can be mild focal or severe Empyema) are common manifestations of invasive disease.

Cellulitis is a bacterial infection involving the skin. It specifically affects the dermis and subcutaneous fat. Signs and symptoms include an area of redness which increases in size over a couple of days. The borders of the area of redness are generally not sharp and the skin may be swollen. While the redness often turns white when pressure is applied this is not always the case. The area of infection is usually painful. Lymphatic vessels may occasionally be involved and the person may have a fever and feel tired.

METHODS AND MATERIAL USED

Epitope prediction

An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. The part of an antibody that recognizes the epitope is called a paratope. Although epitopes are usually non-self proteins, sequences derived from the host that can be recognized are also epitopes. Prediction of antigenic epitopes on protein surface is important for vaccines design or we can say that it is a prediction of protein surface regions that are preferentially recognized by antibodies (antigenic epitopes) can help in the design of vaccines components and immune diagnostic reagents. So from this we can predict the surface of an antigen or a foreign material and through this we can design a particular drug for haemophilus influenza.

Antigenicity

The ability to cause the production of antibodies. The degree of antigenicity of a substance depends on the kind and amount of that substance and on the degree to which the host is sensitive to it and able to produce antibodies also called immunogenicity. Antigenicity is the capability of a chemical structure an antigen to bind specifically with a group of certain products that that have adaptive immunity. Antigenicity was more commonly used in the past to refer to what is now known as immunogenicity.

Immunogenicity:

The property to being able to induce a specific immune response or a degree to which a substance is able to stimulate immune response is called immunogenicity. Or it is the ability of a particular substance such as an antigen or epitope to provoke an immune response in the body of a human or animals. Immunogenicity differentiates in two categories wanted and unwanted. Wanted
immunogenicity is typically related with vaccines where the injection of an antigen (the vaccines) has to lead to an immune response against the pathogen. Unwanted immunogenicity is when organism mounts an immune response against an antigen which is undesired. Unwanted immunogenicity is a strongly linked with therapeutic proteins. A fraction of the patients treated with those drugs mount anti-drug-antibodies.

METHODOLOGY

1. Sequence and Structure comparison:(Blast)
2. CD domain

![Image of NCBI search results for CD domain]

- Graphical summary showing the CD domain
- List of domain hits with their descriptions and locations

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3. Secondary structure Prediction

![GOR4 result for: UNK_179900](image1)

**GOR4 result for: UNK_179900**

- **alpha_helix**: 61 %, 0.708
- **beta_helix**: 12 %, 0.069
- **beta_ribbon**: 11 %, 0.129
- **extended_string**: 8 %, 0.035
- **random coil**: 13 %, 0.036
- **other states**: 0 %

![Secondary structure prediction](image2)

**Secondary structure prediction**

- **alpha_helix**: 61 %, 0.708
- **beta_helix**: 12 %, 0.069
- **beta_ribbon**: 11 %, 0.129
- **extended_string**: 8 %, 0.035
- **random coil**: 13 %, 0.036
- **other states**: 0 %

**Prediction results file [here](image3)**
4. Functional analysis

Protparm (Hydropathy)

| Amino Acid | Frequency | Percentage |
|------------|-----------|------------|
| Ala (A)    | 27        | 5.1%       |
| Arg (R)    | 23        | 4.4%       |
| Asn (N)    | 46        | 8.7%       |
| Asp (D)    | 32        | 6.1%       |
| Cys (C)    | 0         | 0.0%       |
| Gln (Q)    | 20        | 3.8%       |
| Glu (R)    | 25        | 4.8%       |
| Gly (S)    | 62        | 11.8%      |
| His (H)    | 17        | 3.2%       |
| Ile (I)    | 30        | 5.7%       |
| Leu (L)    | 23        | 4.4%       |
| Lys (K)    | 36        | 6.8%       |
| Met (M)    | 7         | 1.3%       |
| Phe (F)    | 23        | 4.4%       |
| Pro (P)    | 20        | 3.8%       |
| Ser (S)    | 33        | 6.3%       |

Molecular weight: 58159.3

Theoretical pI: 8.31
5.1 Geno 3D

5.2 Gene scan
5.3 Gene mark
6. A. Phylogenic analysis (DNA sequence)

![Image of phylogenetic analysis (DNA sequence)]

Figure 1: Phylogenetic tree.

6. B. Phylogenic analysis (Protein sequence)

![Image of phylogenetic analysis (Protein sequence)]

Figure 1: Phylogenetic tree.
7. A. Immunogenicity prediction (Hap protein)
8. A. Immunogenicity prediction (D15 protein)

![Immunogenicity predictions - Prediction Results](image)

8. B. Immunogenicity prediction (D15 protein)

![MHC-I Binding Prediction Results](image)
9. A. Immunogenicity prediction (Htra protein)

| Peptide          | Length | Score  |
|------------------|--------|--------|
| LG4519896        | 15     | 0.257212|
| M6X54477         | 8      | 0.313896|
| SEAKF3185        | 8      | 0.348306|
| M6X54476         | 10     | 0.319740|
| LG451985         | 7      | 0.413355|
| LG451986         | 9      | 0.319246|
| LG451987         | 10     | 0.322780|
| LG451988         | 8      | 0.306884|
| LG451989         | 12     | 0.343444|
| SED4421         | 7      | 0.333333|
| VSY6910         | 7      | 0.333333|
| LG451979         | 7      | 0.307742|
| LG451976         | 9      | 0.307742|
| LG451974         | 7      | 0.300000|
| LG451975         | 11     | 0.392694|
| LG451977         | 7      | 0.380909|
| LG451978         | 24     | 0.153741|
| LG451980         | 11     | 1.154200|
| LG451981         | 8      | 0.977200|
| LG451982         | 21     | 0.639803|
| LG451983         | 21     | 0.631855|
9. B
RESULT

Generalized study of sequence and structure comparison studies, To find out which is the best disease causing target antigens we perform epitope prediction for binding site analysis among the predicted antigens which is showing best antigenicity and immunogenicity score, basing on propensity values for antigenicity and Immunogenicity values and ranking of immunogenicity considered, we select the best targets of HPV type b strains. Among the all selected antigens, Hap, HtrA 1.0024 are showing best Antigencity and also showing best Immunogenecity Hap (immunogencity score(IM score) 0.64 for 48 residues ; P-value -1.22023e).

CONCLUSION

Cancer vaccines in cancer therapies is called immunotherapy which is done either by specific cancer vaccine or universal cancer vaccine which contain tumor antigens that stimulate the immune system which in turn initiate various mechanisms that terminate tumor cells and prevents recurrence of these tumors. Here best antigens are identified these target antigens may helpful for further studies and there may be scope to develop new drugs which cal bitterly interact with selected targets. In these studies finally three best targets are identified as specified in result part.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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