Antibody responses to linear and conformational epitopes

PhD course:
Biological Sequence Analysis
30.05.2008

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Outline

- Antibodies and B-cell epitopes
- Classification of B-cell epitopes
- Prediction methods for B-cell epitopes
  - Presentation of CBS prediction methods
- Examples of B-cell epitopes in vaccine design
Antibodies and B-cell epitopes

Variable CDRs of Fab fragments bind specifically to pathogen epitopes

Conserved Fc fragments are recognizable for macrophages and complement system

1IGT
Harris et al. 1997
Antibodies and vaccines

- Vaccines introduce parts of the pathogen (epitopes) to the immune system

- The immune system then develops antibodies that bind the specific pathogen and which stay in the individual

- Antibodies help to prevent the infection of the pathogen and the individual is immune
B-cell epitopes

- B-cell epitopes are exposed parts of a pathogen molecules which antibodies are developed to recognize and bind

- They are consisting of atoms in a specific spatial arrangement

- Protein B-cell epitopes are classified into linear and discontinuous epitopes

- ~90% of epitopes in globular proteins are discontinuous (Thornton et. al. 1986, Barlow et al. 1986)
Discontinuous B-cell epitopes

An example: An epitope of the Outer Surface Protein A from *Borrelia Burgdorferi* (1OSP)

SLDEKNSVSVDLPGEM
KVLVSKENKDKGKYDLI
ATVDKLELKGTSDKNN
GSGVLEGVKA DK CKVK
LTISDDL GGQTTLVFKE
DGKTLVSDKKVT SKDKS
STEKFN EKGEVSEKI IT
RADGTRLEY TGIKSDGS
GKAKEVLKG

1OSP, Li et al. 1997
Characteristics of binding

- Salt bridges
- Hydrogen bonds
- Hydrophobic interactions
- Van der Waals forces

Binding strength

- Highly flexible amino acid side chains
- Conformational rearrangements upon binding
- “Induced fit” model of interactions
B-cell epitope data bases

- Databases:
  - AntiJen
  - IEDB
  - BciPep
  - Los Alamos HIV database
  - Protein Data Bank
- Large data set: linear epitopes
- Small data set: discontinuous epitopes
Computational/rational vaccine design

PATHOGEN PROTEIN
KVFGRCERAAAMKRHGLDNYR
GYSLGNWVCAAKFESNF

Rational Vaccine Design
Steps in epitope prediction

• The performance is still low compared to T-cell epitope prediction
• Use as much information about your protein as possible
  – Predicted epitopes
  – Multimerization
  – Glycosylation
  – Transmembrane helices
  – Conformational changes
  – Functional sites
Sequence-based methods for prediction of linear epitopes

- **Protein hydrophobicity – hydrophilicity algorithms**
Parker, Fauchere, Janin, Kyte and Doolittle, Manavalan Sweet and Eisenberg, Goldman, Engelman and Steitz (GES), von Heijne

- **Protein flexibility prediction algorithm**
Karplus and Schulz

- **Protein secondary structure prediction algorithms**
GOR II method (Garnier and Robson), Chou and Fasman, Pellequer

- **Protein “antigenicity” prediction:**
Hopp and Woods, Welling

```
TSQDLSVFPLASCKDNIASTSVTLGCLVTGLYPMSTTTVTDGSLNKVINVTFTPFTTHETYGLHSIVSQVTASGKWQFTSGCVHAESTAINKTFSCALNFIPPTVKLHSSCNPGDTHTIIQLLCLISGYVPGDMEVIWLVDGQKATNIFPYTAPGTEGNVTSTHSELNITQGEWVSQKTYTCQVTYQGFTFKDEARKCESDSPRGVTSYLSPPSPL
```
Propensity scales: The principle

- The Parker hydrophilicity scale
- The values describe the hydrophilicity of each amino acid
- Derived from experimental data

Parker et al. 1986, Biochemistry
Propensity scales

(\[-2.78 + (-1.27) + 2.46 + 1.86 + 1.26 + 0.87 + 0.3\] / 7 = 0.39

Example of B-cell epitope predictions using the Parker hydrophilicity scale on the sperm whale myoglobin sequence.

Epitopes are shown in blue. (Pellequer et al. 1993)
Sensitivity and specificity

Sensitivity: A measure of how many annotated epitopes were predicted as epitopes

Specificity: A measure of how many annotated non-epitopes were predicted as epitopes

X% sensitivity
Y% specificity
Evaluation of propensity scales

- Blythe and Flower performed an extensive study of propensity scales for B-cell epitope prediction

- Conclusion:
  - Basically all the classical scales perform close to random!
  - More advanced methods are needed

Blythe and Flower, 2005
DiscoTope

Prediction of residues in discontinuous B-cell epitopes using protein structure information

Haste Andersen et al., Protein Science, 2006
B-cell epitope propensity scale

Frequencies of amino acids in epitopes compared to frequencies of non-epitopes

Several discrepancies compared to the Parker hydrophilicity scale

The DiscoTope method was shown to predict better than the Parker scale alone

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Parker</th>
<th>Log-odds Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>2.460</td>
<td>0.691</td>
</tr>
<tr>
<td>E</td>
<td>1.860</td>
<td>0.346</td>
</tr>
<tr>
<td>N</td>
<td>1.640</td>
<td>1.242</td>
</tr>
<tr>
<td>S</td>
<td>1.500</td>
<td>-0.145</td>
</tr>
<tr>
<td>Q</td>
<td>1.370</td>
<td>1.082</td>
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<tr>
<td>G</td>
<td>1.280</td>
<td>0.189</td>
</tr>
<tr>
<td>K</td>
<td>1.260</td>
<td>1.136</td>
</tr>
<tr>
<td>T</td>
<td>1.150</td>
<td>-0.233</td>
</tr>
<tr>
<td>R</td>
<td>0.870</td>
<td>1.180</td>
</tr>
<tr>
<td>P</td>
<td>0.300</td>
<td>1.164</td>
</tr>
<tr>
<td>H</td>
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<td>1.098</td>
</tr>
<tr>
<td>C</td>
<td>0.110</td>
<td>-3.519</td>
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<tr>
<td>A</td>
<td>0.030</td>
<td>-1.522</td>
</tr>
<tr>
<td>Y</td>
<td>-0.780</td>
<td>0.030</td>
</tr>
<tr>
<td>V</td>
<td>-1.270</td>
<td>-1.474</td>
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<tr>
<td>M</td>
<td>-1.410</td>
<td>0.273</td>
</tr>
<tr>
<td>I</td>
<td>-2.450</td>
<td>-0.713</td>
</tr>
<tr>
<td>F</td>
<td>-2.780</td>
<td>-1.147</td>
</tr>
<tr>
<td>L</td>
<td>-2.870</td>
<td>-1.836</td>
</tr>
<tr>
<td>W</td>
<td>-3.000</td>
<td>-0.064</td>
</tr>
</tbody>
</table>

*Amino acids are listed with descending hydrophilicity using the values of the Parker scale.
Surface exposure and structural protrusion can be measured by residue contact numbers
DiscoTope: Prediction of Discontinuous epiTopes using 3D structures

A combination of:
- Sequentially averaged epitope propensity values of residues in spatial proximity
- Contact numbers

-0.145
+0.691 +0.346 +1.136 +1.180 +1.164
+0.346 +1.136
Sum of log-odds values

Contact number: K 10

DiscoTope prediction value
The DiscoTope web server

www.cbs.dtu.dk/services/DiscoTope

http://tools.immuneepitope.org/stools/discotope/discotope.do
BepiPred

Prediction of linear epitopes using protein sequence information

Larsen et al., Immunome Res. 2006
# The BepiPred method

- Parker hydrophilicity scale
- Markow model: linear epitopes from the AntiJen database
- BepiPred is a combination method

<table>
<thead>
<tr>
<th>Sequence bepipred-1.0b epitope</th>
<th>17 17 0.094 . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence bepipred-1.0b epitope</td>
<td>18 18 0.780 . . E</td>
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<tr>
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<td>19 19 1.013 . . E</td>
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<td>20 20 1.221 . . E</td>
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<td>22 22 1.047 . . E</td>
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<td>23 23 0.817 . . E</td>
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<td>Sequence bepipred-1.0b epitope</td>
<td>28 28 0.336 . . .</td>
</tr>
<tr>
<td>Sequence bepipred-1.0b epitope</td>
<td>32 32 0.106 . . .</td>
</tr>
<tr>
<td>Sequence bepipred-1.0b epitope</td>
<td>33 33 −0.007 . . .</td>
</tr>
</tbody>
</table>

Works with a prediction threshold
The BepiPred web-server

www.cbs.dtu.dk/services/BepiPred

http://tools.immuneepitope.org/tools/bcell/iedb_input
BepiPred prediction in VAR2CSA

- BepiPred performs better than classical propensity scale methods
- BepiPred predicted epitopes successfully in the DBL3X domain of VAR2CSA which is a potential candidate for a vaccine against pregnancy associated malaria.

*Dahlbäck et al 2006, Plos Pathogens*
B-cell epitopes in vaccines

Linear epitopes, presented in peptides

- Easily synthesized and stored
- Problems
  - MHC class II helper-epitope
  - Immunogenicity
  - Multiple conformations
  - Peptidase-degradation
B-cell epitopes in vaccines

• Discontinuous epitopes
  – Presented in subunit vaccines, mimotopes or protein scaffolds
  – Recent projects:
    • OspA a lyme disease vaccine candidate
      – Koide et al. applied structure-based drug design to remove 45% of the protein -> use engineered, stabilized form for vaccines
    • HIV gp120
      – Zhou et al. made a stable, functional conformation for induction of the neutralizing antibody b12

Summary I

- B-cell epitopes are on the surface of antigens
- Single propensity scales are less useful for prediction
- DiscoTope: Structure based prediction of discontinuous epitopes
- BepiPred: Sequence based prediction of linear epitopes
Summary II

- Rational vaccine design based on B-cell epitopes is still in development stage

- Linear epitopes most easily used in vaccine design

- Discontinuous epitopes are harder to use in vaccine design
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