In the name of God

The Rh System

Speaker

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The D Antigen and its Historical context

Rh Positive and Rh Negative
Presence of D Antigen Rh+
Absence of D Antigen Rh-
Discovery
1939 Levine & Stetson HDN
1940 Landsteiner and Wiener
Immunizing Rabbit by
RBC of Rhesus monkeys
Clinical Significance

1) After the A and B Antigens D is the most important RBC Antigen in Transfusion Practice
2) Hemolytic disease of newborn
3) Genetic and genotyping
Other Important Antigens

- Four additional antigens
  C, E, c, e
- Five principal antigen
  D, C, E, c, e and
  We have
- Five antibodies
  Anti – D, Anti – C , Anti – E, Anti – c , Anti – e
  We have no Anti-d
### Determination of some Rh phenotypes from the results of tests with the five principal Rh antisera

<table>
<thead>
<tr>
<th>Anti – D</th>
<th>Anti – C</th>
<th>Antisera</th>
<th>Anti – e</th>
<th>Phenotypes</th>
<th>Rh- hr*</th>
<th>CDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>(R_1)</td>
<td>CcDe</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>(R_1)</td>
<td>CDe</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(R_1 R_2)</td>
<td>CcDEe</td>
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<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>(R_0)</td>
<td>cDe</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(R_2)</td>
<td>cDE</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>(R_2 R_1)</td>
<td>CcDEe</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>(R_z)</td>
<td>CcDE</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>(r)</td>
<td>ce</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>(r')</td>
<td>Cce</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>(r'')</td>
<td>cEe</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(r'r'')</td>
<td>CcEe</td>
</tr>
</tbody>
</table>

* Shorthand terminology
tippett’s prediction
Two closely – linked structural loci on chromosome 1
Determine production of Rh antigen

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>DD</td>
<td>Dd</td>
<td>dd</td>
</tr>
<tr>
<td>CC</td>
<td>Cc</td>
<td>cc</td>
</tr>
<tr>
<td>EE</td>
<td>Ee</td>
<td>ee</td>
</tr>
</tbody>
</table>

Next table : shows the most common combination’s of antigens expressed as haplotypes
Frequencies of the principal Rh Genes (or Gene Complexes)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Gene Combination</th>
<th>Fisher-Race Terminology</th>
<th>Antigenic Specificities</th>
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</thead>
<tbody>
<tr>
<td>( R^1 )</td>
<td>CDe</td>
<td></td>
<td>C,D,e</td>
</tr>
<tr>
<td>r</td>
<td>cde</td>
<td></td>
<td>c,e</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>cDE</td>
<td></td>
<td>c,D,E</td>
</tr>
<tr>
<td>( R^0 )</td>
<td>cDe</td>
<td></td>
<td>c,D,e</td>
</tr>
<tr>
<td>r'</td>
<td>Cde</td>
<td></td>
<td>C,e</td>
</tr>
<tr>
<td>r''</td>
<td>cdE</td>
<td></td>
<td>c,E</td>
</tr>
<tr>
<td>( R^z )</td>
<td>CDE</td>
<td></td>
<td>C,D,E</td>
</tr>
<tr>
<td>( r^y )</td>
<td>CdE</td>
<td></td>
<td>C,E</td>
</tr>
</tbody>
</table>
Figure 1. Schematic representation of RHD, RHCE, and RHAG genes and RhD, RhCE, and RhAg proteins. ◦ on RhD represents amino acid differences between RhD and RhCE. ◦ on RhCE indicates the critical amino acids involved in C/c and E/e antigen expression.
CDE and Rh Antibodies

- The reaction patterns of various RBC Tested with the principal antisera.
  Anti-D
  Anti-C
  Anti-E
  Anti-c
  Anti-e
- Routine pretransfusion studies include only tests for D
- Other antisera are used principally in the resolution of antibody problems or in family studies
Weak expression of D (D^u)

- Not all D+ rbc samples react equally well with every anti – D
- Some D+ rbc may not be directly agglutinated by anti – D
- Additional testing may be required to demonstrate the presence of weak D antigen
Du or weak D

Du phenotypes can arise from several different genetic circumstances.

1- weak D antigen
2- R¹ (CDe) not possible
3- R² (cDE) not possible
4- CDe/Cde (R¹/r)
5- Cde/cDe
6- Sub unite of D. antigen or epitopes

Test for confirmation: antiglobulin test (AHG)
Mechanism of D weak

Cde / Cde  
D negative

\[ \begin{array}{c}
Cde / Cde \\
D negative \\
cDe / Cde \\
Weak D positive \\
cDe / cde \\
D positive
\end{array} \]

Weakened expression of D antigen due to C gene in transposition to D gene.
The number of D Antigen on cell
Classified as D^u

D^u Ce/ D^u cE 450 per cell
D^u ce/dce 290-470 site per cell
D^u ce/dce 110-170 site per cell

Using Flow cytometry showed
Expression of D^u ten times lower
Than D positive
$D_{el}$

$D_{el}$ is a form of D detectable by demonstrating that anti-D can be adsorbed onto and eluted from red cells which do not give other positive serological reactions with anti-D. Probably because the expression of D is enhanced by E in cis. Among the 10% of D negative Japanese were considered to be $D_{el}$. In Hong Kong Chinese was about 30% $D_{el}$. 
Significance of $D^u$ in donors and recipients

- $D^u$ is as a week $D$
- $D^-$ recipients: Only $D^-$ rbc not $D^{u+}$ rbc
- $D^u$ is less immunogenic than $D$ antigen
- HDN: $D^{u+}$ infant and $D^-$ mother
Categories of D

The concept that the D antigen is a mosaic comprising a number of epitopes
- Some people with D+ rbcs
Produced anti – D nonreactive with own rbcs.
- Rho = Rh\textsuperscript{A}, Rh\textsuperscript{B}, Rh\textsuperscript{C}, Rh\textsuperscript{D} sub units
- Tippett 1997 : classified in 9 categories
- Recently more than 30 epitopes ISBT
Deletions of Rh System

- Rare genes exist that encode Rh material lacking activity at the
  E/e
  C/c
  C\textsuperscript{w}D/-
  cD/-
  -D-

Normal (CDE/cde) symbol of CDE
Biochemical Considerations

- The number of Rh antigens present on RBC varies with the Rh phenotype

  $R_1^{rbcs}$
  - 14500-19500 D sites
  - 46000-59500 C sites
  - 18000-24500 e sites

  $R_2^{rbcs}$
  - 16000-33500 D sites
  - 25500 E sites
  - 70000-85000 c sites

Molecular weight for Rh 15-174 KD
Rh null Syndrome

- In the more common regular type of Rh-null, the absence of a very common regular gene $X^1_r$ prevents expression of the normal genes at the Rh locus on chromosome 1.
- There are no Rh antigens on RBC.
- RBC lacking Rh antigens have membrane abnormality.
  
  Hemolysis and
  Anemia
  Stomatocytosis
  Shortened RBC survival
Rh antibodies

Anti-D Anti-C Anti-E Anti-c Anti-e
Anti-CDE
Anti-CD
Anti-Ce

Anti-D Monoclonal
- Type of antibody: IgG rarely IgM (Human)
- Monoclonal IgG is saline Reactive
- Best Temperature for activity is 37c
- Anti – Rh is absent normally in human sera
Association of Rh Glycoprotein with Other Blood Group

- The glycoprotein that bear the LW, Duffy and U antigens, all seem to require the presence of Rh protein for full expression.
- Rh-null cells lack all the LW antigens are negative for Fy\(^5\) of Duffy system.
- The membrane glycoproteins RhAG and CD47 have also been shown to be an integral part of this complex.
Association of Rh Glycoprotein with Other Blood Group

- Rh antigens play a structural role in the red cell membrane as evidence by red cell morphology changes in Rh-null syndrome.
- There is evidence, however, that the RhAG protein plays a role in ammonium transport.
The G Antigen and Cross-Reactions

- The G antigen results from serine at position 103 of the Rh polypeptides and is encoded by either RHD or RHCE.
- As a result, the G antigen is almost invariably present on red cells possessing either C or D.
- Antibodies against G appear superficially to be anti-C+D.
- But the anti-G activity cannot be separated into anti –C and anti – D.
The G Antigen and Cross-Reactions

- The fact that G appears to exist as an entity common to C and D
- The fact that D-negative persons immunized by C-D+ red cells sometimes appear to have made anti-C as well as anti-D
- It may also explain why D-negative persons who are exposed to C+D- red cells may develop antibodies appearing to contain an anti-D
The G Antigen and Cross-Reactions

- Differentiation of anti-D-C and –G is not necessary in the pretransfusion setting
- Because virtually all D-C red cells are G-negative
- In obstetric patients believe it is essential distinguish the antibody specific to determine the need for RhIG prophylaxis
بطور خلاصه نتیجه گیری می‌کنیم که

آنتی‌زن D:

1- ازنظر هماتولوژی اهمیت زیادی دارد ABO
2- این آنتی‌زن از بقیه آنتی‌زناهای سیستم گروه‌های خونی بعد از قوی ترین است.
3- پلی پپتید داخل غشایی این آنتی‌زن دارای وزن مولکولی 32-30 KD
4- مطالعه جدید نشان داده که این پلی پپتید بصورت پنجه‌ای بر روی اسیدهای چرب (Fatty acid) قرار گرفته است.
5- با شناخت سکانس آمینواسیدهای آنتی‌زن D زناهای مربوط به آن تا اندازه ای شناسائی شده است.
6- تا بحال بیش از ۳۰ شاخص آنتی‌ژنی از D شناسایی شده (آنتی‌پدیها) منو کلوننال

7- استفاده از آنتی‌پدی‌های منو کلوننال در تایپ این سیستم مشکل آفرین،

هر آنتی‌پدی می‌تواند منو کلوننال خاص یک آنتی‌ژن (Specific) شناخته شده D منو کلوننال با یکی از شاخص D برای اکتشاف آنتی‌پدی D دیگر و همچنین عدم وجود آنتی‌پدی D از قسمتی از آنتی‌پدی D موجب منفی شدن و مثبت شدن می‌شود.

8- می‌توان گفت می‌باشد دیگر آنتی‌پدی D های آنتی‌پدی D خاص یک آنتی‌پدی D باشد. می‌باشد بهتر استثابت شده که آنتی‌پدی از پدی‌ها یکی کلوننال پرای تایپ سیستم بهتر می‌باشد.

9- ثابت شده که آنتی‌پدی با پدی‌ها یکی کلوننال سالین راکتیو می‌باشد.

10- آنتی‌پدی می‌باشد دیگر آنتی‌پدی انسانی آلیورنی یا راکتیو جنین می‌باشد.

11- آنتی‌پدی پدی می‌باشد دیگر آنتی‌پدی می‌باشد.