Autoantibodies and antibacterial antibodies: from both sides now

This year marks the 100th anniversary of the first description of antibodies by von Behring and Kitasato. Within 10 years Ehrlich had developed the concept of 'horror autoxicus'—the idea that the body's immune system would not permit the production of antibodies binding to self components. In contrast, over the past 90 years it has become apparent that not only does the normal immune system have the potential to produce autoantibodies but it frequently does so. The precise relation between antibodies which bind self antigens and those reacting with foreign epitopes remains to be determined.

One of the primary functions of the immune system is to protect the organism against pathological microbial agents. The immune system must therefore be able to recognise bacterial antigenic determinants in order to mount an effective immune response.

This leader explores the relation between autoantibodies and antibacterial antibodies from both sides. Do they simply represent two sides of the same coin or is there simply a variety of different coins, albeit minted in much the same way?

Anti-self antibodies from healthy subjects
Antibodies against self determinants have been demonstrated in normal human sera as well as in sera from patients with autoimmune and infectious diseases, and blood dyscrasias. Thus Avramaes and colleagues have shown that a serum pool from 800 healthy donors contained antibodies against nine common self antigens—tubulin, actin, thyroglobulin, albumin, myoglobin, cytochrome c, fetuin, transferrin, and collagen. Daar and Fabre examined normal sera for reactivity against tissue extracts from brain, heart, liver, and kidney and found that each serum tested bound to all tissues except kidney.

Anti-DNA antibodies appearing in normal subjects have been described by several groups. Anti-DNA antibody synthesis has been shown after stimulation of normal human lymphocytes by pokeweed mitogen, Epstein-Barr virus, Klebsiella pneumoniae, and even without mitogen stimulation. Pisetsky et al using an in vitro system for B cell stimulation have shown that B cells from normal adult and cord blood can secrete antibodies with rheumatoid factor activity and DNA binding activity in response to anti-CD3, and rheumatoid factors in response to Staphylococcus aureus Cowan I and T cell cytokines. These data suggest that rheumatoid factor and anti-DNA antibody production may be regulated independently. Cord blood and fetal liver B cells transformed by Epstein-Barr virus have been shown to secrete IgM, which binds to a variety of autoantigens, including Fc of IgG, single stranded (ss) DNA, double stranded (ds) DNA, cardiolipin, histones 1-4, collagen types I and II, thyroglobulin, and cytoskeletal components. A number of these antibodies are polyreactive. Some cord blood IgM antibodies also bind to extracts of microorganisms, including Staphylococcus aureus, streptococcus, mycobacteria species, and candida. Interestingly, two antibodies bound both to ssDNA and Staphylococcus aureus. Similarly, murine anti-DNA antibodies derived from MRL/lpr mice can bind to Streptococcus faecalis, and to phospholipids extracted from the bacterial cells. This binding could be inhibited by polysaccharides.

Human monoclonal antibodies have been produced by fusing a human lymphoblastoid cell line with normal peripheral blood lymphocytes, normal tonsillar lymphocytes, and normal fetal lymphocytes which bind to DNA, cardiolipin, and platelets. Platelet binding human hybridoma antibodies derived from patients with lupus have greater antigen specificity and are more cytotoxic to platelets than similar antibodies derived from normal subjects.

Thus B cells from normal subjects can produce antibodies with anti-self activity. These antibodies are mostly IgM, probably binding with low affinity to a variety of self antigens. Formal measurements of affinity have been rarely undertaken. Antibodies from cord blood can bind to both endogenous and exogenous antigens. This contrasts with the more selective reactivity and higher affinity of the IgG antibodies, characteristic of a mature antigen driven response.

It is of interest, however, that studies of the cross reactivity of idiotypically related anti-DNA antibodies which were affinity purified from the serum of normal subjects and patients with lupus, and eluted from the kidneys of patients with lupus, have suggested that those from normal serum are the most specific, only cross reacting with polydeoxythymidylic acid, whereas those eluted from kidney cross reacted with a range of synthetic polynucleotides and phospholipids. These data indicate that polyreactive antibodies may have a role in the pathogenesis of tissue lesions.

Antibody affinity and reactivity
The precise relation between polyreactive low affinity autoantibodies and high affinity monoreactive antibodies remains unclear. It is uncertain whether these groups of
antibodies are derived from the same pool of antibodies. Limiting dilution analysis of Epstein-Barr virus transformed CD5+ and CD5− B cells from healthy subjects has shown that the CD5+ B cell population is mainly committed to the production of polyclonal (mainly IgM) antibodies that react with a variety of self antigens.19-21 The CD5− B cell population includes mostly lymphocytes committed to the production of more selectively binding (mainly IgG) antibodies in immunised subjects.19 The CD5+ B cells account for the high incidence of circulating B cells committed to the production of antibodies binding to self and foreign components.19 20

In patients with rheumatoid arthritis the number of CD5+ B cells is increased (27-52%),20 23-24 and a small proportion of these cells secrete high affinity monoreactive rheumatoid factor.20 A similar increase is seen in patients with Sjogren's syndrome.25 26 In patients with systemic lupus erythematosus (SLE), however, no increase in CD5+ B cells is seen and the antibodies produced are mainly polyclonal, but monoreactive high affinity IgG ssDNA autoantibodies are produced by CD5− B cells, present at relatively high frequency, the B cell repertoire of these patients.27 There is evidence to suggest that pathogenic antibodies may exist independently of CD5+ B cells. After in vitro stimulation with lipopolysaccharide CD5+ cells do not, however, preferentially secrete anti-DNA antibodies.28

Recently, Suzuki and colleagues used an enzyme linked spot (ELISPOT) assay to investigate the antibodies secreted by CD5+ and CD5− B cells in patients with SLE and in normal subjects.29 In the patients with SLE both CD5+ and CD5− B cells secreted ssDNA and dsDNA antibodies of both IgM and IgG isotype. Interestingly, they noted IgG antibody secretion by CD5+ B cells. Production of anti-DNA antibodies by CD5− B cells correlates well with polyclonal immunoglobulin production. They suggested that in human SLE there exist two anti-DNA antibody populations, one of which independently secretes anti-DNA antibodies (CD5+) and a second, CD5−, which produces anti-DNA antibodies as a result of polyclonal B cell activation.29

Support for the notion that anti-DNA antibodies arise as a result of polyclonal B cell activation comes from data showing that cultured lymphocytes from patients with lupus not only secrete anti-DNA antibodies but also antibodies against common environmental antigens, such as influenza virus haemagglutinin, adenovirus hexon and mannin from Candida albicans.30

Autoantibodies in infectious diseases and dyscrasias
Autoantibodies are also produced during the course of infectious disease, in particular mycobacterial and Gram negative infections. Antinuclear antibodies, rheumatoid factors, and antithyroglobulin antibodies were initially reported in the sera of patients with chronic pulmonary tuberculosis,31 but many of these patients were taking drugs which can induce a lupus like syndrome. Subsequently, Sela and colleagues reported the presence of anti-DNA and antcardiolipin antibodies in patients with active untreated tuberculosis.32 Anti-DNA antibodies have been reported in the sera of up to 27% of patients with klebsiella infections.33 Isenberg and colleagues have reported that rheumatoid factors, antinuclear antibodies, and anti-poly(ADP-ribose) antibodies are present in 15-40% of sera from patients with active untreated tuberculosis, klebsiella septicaemia, klebsiella, and Escherichia coli urinary tract infections.34 Furthermore, sera from lupus patients have been shown to contain anti-klebsiella antibodies, which cross react with anti-DNA antibodies.35

Recently, it has been suggested that there are IgG subclass differences between autoantibodies and antipoly-saccharide antibodies. DNA binding antibodies in SLE are predominantly IgG1 and IgG3,36 whereas antipoly saccharide antibodies are IgG2.37 This may result in functional differences between antibodies. The monoclonal gammapathies multiple myeloma and Waldenstrom's macroglobulinaemia permit the study of naturally occurring monoclonal immunoglobulins. It has been established by several groups that these antibodies may have rheumatoid factor activity with a prevalence of about 16%.38-40 Binding to dsDNA occurs in 5-10%,51-43 In addition, binding to a variety of synthetic polypeptides,44 histones, and, rarely, to mitochondria, gastric parietal cells cytoskeletal components, and thyroid determinants has been described.41 45 We have recently tested a panel of 75 myeloma sera for a wide range of autoantibodies and found that 22% possessed autoactivity.46 Sestak et al studied the occurrence of antiRo/SS-A, anti-La/SS-B, and anti-RNP activity in a panel of 143 paraprotein containing sera and found activity in 3.5%, 0-7%, and 0% respectively using a precipitation method.45 It should be stressed that in most cases it was not formally proved that it was the myeloma protein that carried autoactivity and not some other protein constituent of serum. Kabat et al have described a human monoclonal macroglobulin with specificity against (2→8)-linked poly-N-acetylneuraminic acid (the capsular polysaccharide of group B meningococci and E coli K1) which cross reacts with polynucleotides and ssDNA.48

Several examples have now been described of cross reactivity between DNA binding antibodies and a variety of bacterial antigens, which may result in disturbances of immune function and lead to the appearance of autoantibodies. The light chain of the human DNA binding monoclonal antibody 16/6 has been shown to have an amino acid sequence homology (39 out of the first 40 residues) with a Waldenstrom monoclonal IgM (designated WEA) which binds a Klebsiella pneumoniae cell wall polysaccharide called K30.49

Idiotype considerations
Further evidence of a link between autoantibodies and antibacterial antibodies comes from the study of the idiotype cross reactions. The public DNA antibody idiotype 16/6 (first described on human monoclonal IgM antibody 16/6) is present on the WEA protein.50 Studies of the sera from patients with klebsiella infections have shown that 37% have high titres of the 16/6 idiotype.33 In two patients whose sera had high anti-K30 activity and 16/6 idiotype levels but no DNA binding activity the anti-K30 and 16/6 idiotype levels could both be reduced by absorption against K30.33 This strongly suggests that in non autoimmune subjects a common DNA idiotype is present on an antibacterial antibody.

A similar idiotype relation may exist between DNA antibodies and mycobacterial antibodies. Monoclonal anti-Mycobacterium tuberculosis react with DNA and can act as antinuclear antibodies. Some of these antibodies carry the 16/6 idiotype.51 Recently, two DNA binding monoclonal antibodies (PR4 and TH3) have been described, which were derived from the peripheral blood lymphocytes of patients with leprosy.52 Both PR4 and TH3 bind to PGL-1 (a unique M leprae glycolipid) as well as to ssDNA and dsDNA. Using a panel of human monoclonal antibodies and their homologous anti-idiotypes it has been shown that there is a high degree of idiotype sharing between lupus derived antibodies—for example 16/6, 21/28, which bind DNA, and leprosy derived antibodies—for example 8E7, TH9, which do not.53

Serum studies have shown that the common idiotypes
PR4, 16/6, and B2G (BEG 2 was first described on a monoclonal DNA binding antibody derived from human fetal lymphocytes\(^\text{16}\)) can be detected in the serum of patients with active untreated tuberculosis, leprosy\(^\text{32}\) (Watts, unpublished data; Zumla, unpublished data), and Lyme disease (Axford, Watts, Williams, unpublished data) as well as in the serum of patients with SLE\(^\text{16}\) \(^\text{54}\) \(^\text{55}\) (table 1). The leprosy derived idiotypes 8E7 and TH9 are present in the serum of patients with Lyme disease, SLE, and in normal subjects\(^\text{36}\) (table 1). The 16/6 idiotype has also been found in the serum of patients with the parasitic infections schistosomiasis and filariasis.\(^\text{57}\) There was no relation between the presence of the 16/6 idiotype and anti-DNA antibodies levels or the occurrence of nephritis as a complication of helminthic infections in most cases, however, it has not been proved formally that the cross reactive idiotypes are present on antibacterial antibodies in serum.

### Sequencing of autoantibodies

Over the past five years the nucleotide sequence of the heavy and light chains of a number of human monoclonal antibodies has been determined. It is now clear that they can be encoded for by unmutated germline genes.\(^\text{58-63}\) The \(V_H\) genes of anti-DNA antibodies have been shown to be encoded for by members of the \(V_{HI}, V_{HII}, V_{HIII},\) and \(V_{HIV}\) families \(^\text{58}\) \(^\text{61}\) \(^\text{63}\) (Watts, Hillson, and Isenberg, unpublished data) (table 2). Analysis of a panel of 11 human hybridoma monoclonal rheumatoid factors has shown that most were encoded for by \(V_{HII}\) and two by \(V_{HI}.\)\(^\text{64}\)

An anti-Sm antibody has been shown to belong to the \(V_{HIII}\) family.\(^\text{65}\) Sanz et al. sequenced eight autoantibodies derived from normal subjects and found that one each were from the \(V_{HI}, V_{HII}, V_{HIII},\) and \(V_{HIV}\) families.\(^\text{58}\) \(^\text{61}\) \(^\text{63}\) The overrepresentation of the \(V_{HIII}\) family is not surprising as it is believed to be the largest \(V_H\) family, but the \(V_{HIV}\) family is much smaller.\(^\text{62}\) Clearly at present only a small number of antibodies have been sequenced and the apparently biased \(V_H\) gene usage may not be confirmed when larger numbers of antibodies have been sequenced. The occurrence of cross reactive idiotypes on antibodies of different specificities suggests that a restricted set of germline genes which have been conserved encode for autoreactivity.

Further evidence suggesting a close link between auto-antibodies and antibacterial antibodies comes from the observation that a single amino acid mutation in the \(V_H\) gene of a monoclonal antiphosphocholine antibody (S107) results in a loss of ability to bind to phosphocholine and acquisition of the capacity to bind to DNA.\(^\text{66}\) Eilat has shown amino acid sequence homology between the \(V_H\) region of an NZB/NZW monoclonal anti-dsDNA antibody and an antibody against phosphocholine.\(^\text{67}\)

### Origins of autoimmunity

Holmgren has argued that the natural antibodies with self reactivity, polyreactivity, and a high degree of connectivity through the idiotype network are important in the development of self tolerance.\(^\text{68}\) Through evolutionary selection, antibody \(V\) regions with these properties are maintained and expressed early in ontogeny. These naturally occurring activated lymphocytes make up 10–15% of the total lymphocyte population and may be responsible for maintenance of normal network dynamics and self tolerance.

The above data go some way towards answering the question whether an antibody with anti-self activity may, under antigen drive, become specific for anti-foreign activity or if an anti-foreign antibody may develop autoreactivity.

Changes in antigen binding specificity occurring during affinity maturation and somatic mutation have been studied in the arsonate system in normal A/J mice. In the preimmune animal a high proportion of the antibodies of the arsonate system react with DNA and cytoskeletal components. After immunisation the affinity for hapten increases and reactivity with autoantigens is lost. A few antibodies retain some autoreactivity together with anti-arsonate activity. Competition assays have suggested that the same V region site binds to both arsonate and DNA. The changes in affinity were due to somatic mutation of the \(V_{IDC}\) (a major idiotype of the arsonate system).\(^\text{69}\) These data suggest that autoreactivity is a feature of the pre-immune system, which is lost during affinity maturation and somatic mutation.

In contrast, Weigert and colleagues have elegantly dissected the genetic origin of anti-DNA antibodies and rheumatoid factors in the lupus prone MRL/lpr mouse, showing that autoantibodies appear as a result of oligoclonal B cell expansion and that such expansion is specific and antigen receptor driven.\(^\text{70}\) \(^\text{71}\) In the mouse, as in man, the preimmune repertoire includes mainly ssDNA specific B cells with few dsDNA specific B cells.\(^\text{72}\) \(^\text{73}\) Shlomchik found ssDNA and dsDNA specific B cells that were descended from the same precursor B cell, suggesting that somatic mutations can cause specificity for dsDNA and that such antibodies are selected for.\(^\text{74}\) The \(V_H\) sequences of anti-DNA antibodies which share an idiotype may be derived from different \(V_H\) and \(V_{L}\) families.\(^\text{71}\) \(^\text{73}\) Whether this is also true in man remains to be seen.

On balance it now seems likely that native DNA antibodies arise by somatic mutation under antigen drive. The polyreactive antibodies identified in normal human serum may well play a major part in vivo as a first line of defence against invading micro-organisms. The polyreactive human monoclonal hybridoma antibodies are probably representative of these antibodies. Having a relatively poor fit for surface antigens on the micro-organism and low affinity they are unable to eliminate the invaders completely. This process is completed by the high affinity monoreactive (IgG) antibodies that appear as a result of antigen driven.

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**Table 1** Common DNA idiotypes in infectious diseases. Values are shown as percentages

<table>
<thead>
<tr>
<th></th>
<th>166</th>
<th>BEG 2</th>
<th>PR4</th>
<th>8E7</th>
<th>TH9</th>
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<tbody>
<tr>
<td>Normal subjects</td>
<td>4(^\text{11})</td>
<td>7(^\text{16})</td>
<td>3(^\text{15})</td>
<td>2(^\text{16})</td>
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<td>Syphilitic lymphogranulomas</td>
<td>4(^\text{11})</td>
<td>8(^\text{16})</td>
<td>7(^\text{16})</td>
<td>4(^\text{16})</td>
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<td>Tuberculosis</td>
<td>6(^\text{10})</td>
<td>3(^\text{4})</td>
<td>3(^\text{7})</td>
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<td>NK</td>
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<tr>
<td>Leprosy</td>
<td>NK</td>
<td>4(^\text{4})</td>
<td>5(^\text{9})</td>
<td>3(^\text{8})</td>
<td>4(^\text{4})</td>
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<td>Klebsiella</td>
<td>37(^\text{1})</td>
<td>14(^\text{1})</td>
<td>NK</td>
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<td>NK</td>
</tr>
<tr>
<td>Non-klebsiella</td>
<td>25(^\text{1})</td>
<td>15(^5)</td>
<td>NK</td>
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<td>Filariasis</td>
<td>35(^\text{1})</td>
<td>6(^\text{2})</td>
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<tr>
<td>Schistosomiasis</td>
<td>24(^\text{1})</td>
<td>NK</td>
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<tr>
<td>Lyme disease</td>
<td>65(^\text{1})</td>
<td>60(^\text{1})</td>
<td>60(^\text{1})</td>
<td>15(^\text{5})</td>
<td>15(^\text{5})</td>
</tr>
</tbody>
</table>

NK = not known.

\(^{\text{a}}\)Zumla et al., unpublished data.

\(^{\text{b}}\)Watts et al., unpublished data.

\(^{\text{c}}\)Axford et al., unpublished data.

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**Table 2** V gene usage in human DNA monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>(V_H)</th>
<th>(V_{L})</th>
<th>(V_{L})</th>
<th>Reference</th>
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<tr>
<td>BEG 2</td>
<td>4</td>
<td>(_)</td>
<td>NK</td>
<td>*</td>
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<tr>
<td>I2/1</td>
<td>1</td>
<td>1</td>
<td>(_)</td>
<td>58</td>
</tr>
<tr>
<td>I2/28</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>58</td>
</tr>
<tr>
<td>HE10</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>TH3</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>61</td>
</tr>
<tr>
<td>I2/17</td>
<td>3</td>
<td>1</td>
<td>(_)</td>
<td>58, 63</td>
</tr>
<tr>
<td>Km 4-6</td>
<td>3</td>
<td>NK</td>
<td>NK</td>
<td>60</td>
</tr>
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<td>CB82</td>
<td>4</td>
<td>NK</td>
<td>NK</td>
<td>59</td>
</tr>
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NK = not known.

\(^{\text{a}}\)Watts, Hillson, and Isenberg, unpublished data.
somatic mutation or cell lines that use new Vβ/Vδ combinations. The work of Casali and colleagues has suggested that the polyclonal autoantibodies differ functionally from the autoantibodies characteristic of autoimmune disease with more restricted reactivity and higher affinity, whereas Suzuki has produced evidence suggesting that there are two populations of anti-DNA antibodies derived from different cell lineages. These antibodies are produced by cells that are generally confined to patients with autoimmune conditions. Shlomchik's experiments indicate that in the mouse, as in the case of the response to exogenous antigen, anti-DNA antibodies and rheumatoid factors characteristic of autoimmune disease occur as a result of antigen driven somatic mutation.

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