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THE ORIGIN OF AUTOANTIBODIES

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SUMMARY

Generally two types of autoantibodies are discriminated: "Natural autoantibodies" and "pathogenetic autoantibodies". Natural autoantibodies are normal components of the immune system. They seem not to account for autoimmune disease. These antibodies usually are of the IgM class and of low affinity. Pathogenetic autoantibodies, on the other hand, generally are of the IgG class and of high affinity. These autoantibodies are potentially harmful.

This paper presents data on the role of environmental antigens in the production of antibodies and especially autoantibodies. We made use of germfree mice fed chemically defined synthetic diet (GF-CD mice). These mice have a seemingly normal IgM production and a severely reduced production of IgG and IgA. The specificity repertoire of these IgG and IgA antibodies greatly differs from that of the same isotypes in conventional mice, and is much alike the IgM specificity repertoire. The GF-CD mice also differ from conventional mice in their higher production of natural autoantibodies. Apparently, exogenous antigenic stimulation plays an important role in the development of the actual B-cell repertoire.

Exogenous antigenic stimulation, especially infectious disease, can account not only for autoantibodies, but can also facilitate autoimmune disease. Several mechanisms have been proposed, including (a) change in endogenous antigen; (b) disturbance of the host immune response; (c) molecular mimicry; and (d) somatic diversification of antibodies to environmental antigens. Literature data supporting the latter two possibilities are discussed.

THE INFLUENCE OF ENVIRONMENTAL ANTIGENS ON IMMUNOGLOBULIN PRODUCTION

We studied the influence of environmental antigens on the immunoglobulin (Ig) production by comparing germfree mice fed with a chemically defined synthetic diet (GF-CD mice) and conventional mice fed natural ingredient diet (CV-NI mice). The GF-CD mice were bred and maintained by Drs. B.S. Wostmann and J.R. Pleasants from the Lobund Laboratory in Notre Dame, Indiana. The chemically defined diet used, consisted of sugars, amino acids, lip-

Table 1: Numbers of "background" Ig-secreting cells in spleen, bone marrow and mesenteric lymph nodes of "antigen-free" (GF-CD) and conventional BALB/c mice (CV-NI)[a]

Organ	Isotype	Ig-SC x 10^{-3}/organ GF-CD	CV-NI	Ratio CV-NI/GF-CD
Spleen	IgM	267 ± 50[b]	469 ± 75	1.8 (p>0.1)[c]
	IgG1	0.1 ± 0.1	25 ± 5	250 (p<0.05)
	IgG2a	0.2 ± 0.08	29 ± 6	145 (p<0.05)
	IgG2b	0.2 ± 0.09	20 ± 5	100 (p<0.05)
	IgG3	0.1 ± 0.07	14 ± 3	140 (p<0.05)
	IgA	0.3 ± 0.3	240 ± 41	800 (p<0.01)
Bone marrow	IgM	97 ± 25	61 ± 6	0.63 (p>0.5)
	IgG	2.0 ± 0.4	73 ± 11	37 (p<0.005)
	IgA	1.5 ± 0.7	107 ± 38	71 (p<0.05)
Lymph nodes	IgM	4.6 ± 2.5	4 ± 1.5	0.87 (p>0.1)
	IgG	0.2 ± 0.1	58 ± 18	290 (p<0.05)
	IgA	0.3 ± 0.1	31 ± 2	103 (p<0.001)

[a]Data from: *Bos* et al., 1988.
[b]Numbers represent the arithmetic means (± SEM) of three to seven individual experiments. In each experiment the organs of six animals of each group were pooled.
[c]Statistical analysis was performed with the Student *t* test.

ids, minerals and vitamins, all with a molecular weight lower than 10.000 D (*Pleasants* et al., 1986). Such GF-CD mice are tentatively called "antigen-free mice".

In the lymphoid organs of CV-NI mice many times more Ig-secreting cells (Ig-SC) occur than in GF-CD mice. Table 1 shows the numbers of IgM-, IgG- and IgA-SC in spleen, bone marrow and mesenteric lymph nodes of both groups of mice. While the numbers of IgM-SC hardly differ, the numbers of IgG- and IgA-SC are much lower in GF-CD than CV-NI mice in each of the three organs studied. The table also shows the ratio of the numbers of IgG- and IgA-SC found in CV-NI mice over those in GF-CD mice. In most cases this ratio is 100 or higher, indicating that environmental antigens affect the IgG and IgA production much more than the IgM production.

This conclusion urged for studies on selective pressures accounting for this differential production of different isotypes. Therefore, we analysed the specificity repertoire of the IgM-, IgG- and IgA-SC in both groups of mice. This was done by employing haemolytic plaque assays using sheep red blood cells (SRBC) coated with different haptens and ELISA-plaque assays. Remarkably, no substantial differences were found between GF-CD and CV-NI mice with regard to frequencies of splenic IgM-SC specific for the different antigens (Table 2). Apparently the specificity repertoire of the spontaneously occurring ("background") IgM-SC is rather stable and hardly dependent on exogenous antigenic stimulation.

For IgG and IgA, however, the situation is quite different. In CV-NI mice the frequencies of IgG- and IgA-SC specific for DNP-BSA were found to be substantially lower than the frequency of IgM-SC specific for DNP-

Table 2: Relative frequencies of background IgM-secreting cells specific for several haptens in the spleen of "antigen-free" (GF-CD) and conventional BALB/c mice (CV-NI)[a]

Antigen	GF-CD	CV-NI
NIP_4-SRBC	1 in 46 (±29)	1 in 21 (±4)[b]
$NIP_{0.4}$-SRBC	1 in 195 (±131)	1 in 188 (±85)
NNP_2-SRBC	1 in 63 (±31)	1 in 38 (±11)
$NNP_{0.2}$-SRBC	1 in 284 (±110)	1 in 1125 (±681)
TNP_{30}-SRBC	1 in 232 (±71)	1 in 122 (±36)

[a]Data from: *Bos* et al., 1986.
[b]Figures represent the mean ratio of specific IgM-antibody secreting cells to the total number of IgM-secreting cells as detected in the protein A plaque assay. The arithmetic mean ± SD has been calculated.

BSA. In GF-CD mice such a substantial difference was not found (Table 3). In such "antigen-free" mice the relatively small production of IgG and IgA seems to be independent of the selective antigenic forces that in conventional mice greatly affect the specificity repertoire of the compartments of IgG- and IgA-SC. This observation leads us to suggest that the background Ig-SC can be subdivided into an "antigen-dependent" and an "antigen-independent" compartment.

The antigen-dependent compartment consists of B-cells which, upon activation by exogenous antigens, give rise to primary and secondary humoral immune responses with antigen specific antibody production, memory induction, isotype switch and affinity maturation.

The antigen-independent compartment, in our view, consists of B-cells that are driven to develop into clones of Ig-SC by endogenous stimuli. This compartment consists mainly of IgM-SC, although the low numbers of IgG- and IgA-SC in GF-CD mice also belong to this compartment. The endogenous stimuli may be provided by idiotypes, e.g., from maternal Ig transferred via the placenta or milk (*Bernabé* et al., 1981) or other self-antigens during the development of the immune system (*Steele* and *Cunningham*, 1978). Idiotypic interactions with T-cells might also represent a driving force and/or

Table 3: Frequency of DNP_{27}-BSA-specific Ig-secreting cells in the spleen of "antigen-free" (GF-CD) and conventional BALB/c mice (CV-NI)[a]

	GF-CD		CV-NI	
Ig-secreting cells	Cells tested[b]	Frequency	Cells tested	Frequency
IgM	222	1 in 143 (±32)	52	1 in 65 (±22)
IgG	1.9	1 in 48 (±18)	989	1 in 60000 (±22060)
IgA	1.5	1 in 93 (±37)	465	1 in 2118 (±327)

[a]Data from: *Bos* et al., 1988.
[b]Total number of Ig-secreting cells (x 10^{-3}) of a particular isotype evaluated for specificity for DNP_{27}-BSA.

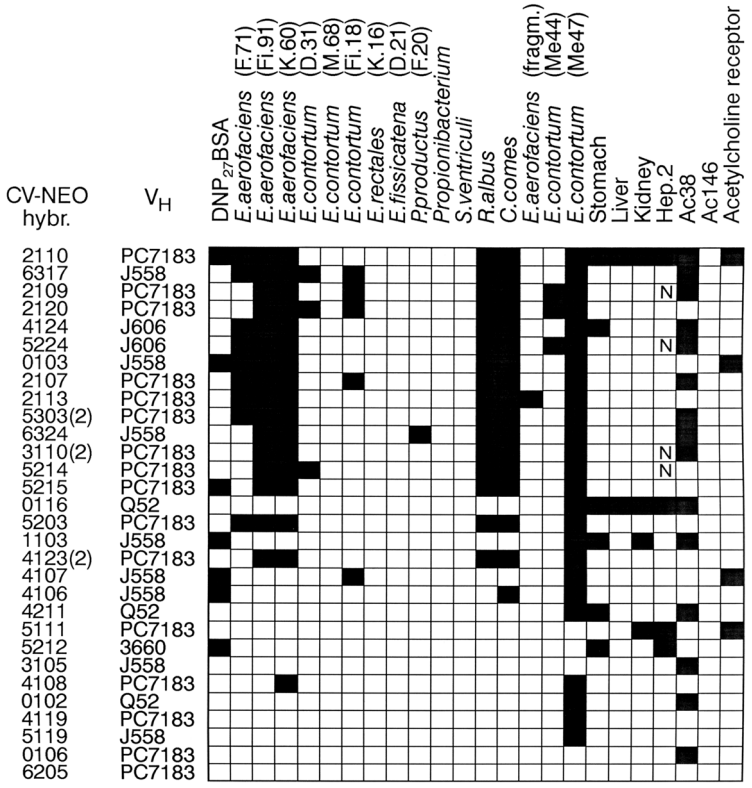

Figure 1: Reactivity pattern and V_H gene usage of a panel of hybridomas from neonatal conventional BALB/c mice. Closed squares represent positive reactions; open squares represent negative reactions; N = not done. Data from: *Bos* et al., 1989.

play a regulating role (*Martinez-A* et al., 1986). This autonomous compartment remains very stable during the lifetime and is relatively independent of exogenous stimuli (cf. Tables 2 and 3). It can be speculated that the B-cells involved belong to the naturally activated, autoreactive B-cells described by *Portnoï* et al. (1986).

NATURAL AUTOANTIBODIES

Many individuals have autoantibodies in their serum. The frequency of people with autoantibodies increases with increasing age (*Hawkins* et al., 1979). These autoantibodies include specificities for neurofilaments, tubulin, actin, transferrin, thyroglobulin etc. (*Guilbert* et al., 1982). The occurrence of autoantibodies is by far not always associated with autoimmune disease. In humans, autoantibodies can even occur for a decade or longer without signs of autoimmune disease. This discrepancy is found not only in humans, but also in mice (*Hawkins* et al., 1979; *Schattner*, 1987).

Highly informative with regard to autoantibody production are studies of hybridoma collections produced by fusing unstimulated or lipopolysaccharide (LPS) stimulated B-cells from naive mice with non-secreting murine plasmacytoma cells. Using this methodology, B-cells with the capacity of se-

creting autoantibodies have been shown to be normal components of the immune system. Such natural autoantibodies are usually of the IgM class and of low affinity (*Holmberg* et al., 1986a).

Early in ontogeny a high frequency of B-cells can be found which can bind to multiple antigens, among which autoantigens (*Dighiero* et al., 1985). Such B-cells have been called "multireactive B-cells". The antibodies produced by many of such B-cells can also recognise different antibody combining sites or determinants specific to Ig variable regions of other B-cells. They are called "highly connective" antibodies and may participate in an idiotypic network (*Holmberg* et al., 1984, 1986b).

In Figure 1 we show cross-reactivity between autoantigens and exogenous antigens for a number of hybridomas derived from unstimulated neonatal spleen cells. Probably, B-cells that produce multireactive, including autoreactive, antibodies persist throughout the mouse' lifetime, but their frequency is higher early in ontogeny (*Holmberg* et al., 1986b). This conversion, however, is much less prominent in the absence of exogenous antigenic stimulation, as shown in adult GF-CD mice (cf. Figure 2). This indicates that exogenous antigenic stimulation plays an important role in the development of the actual B-cell repertoire.

The specificity of Ig molecules is determined by the variable part of the heavy and light chains, which are largely encoded by V_H and V_L gene segments. V_H gene segments have been grouped into at least eight different families (called: PC7183, Q52, X24, 3660, J606, S107, J558, 3609) on the basis of relatedness at the nucleotide sequence level (*Brodeur* and *Riblet*, 1984; *Winter* et al., 1985). The V_H genes within a family are highly homologous, with more than 80% sequence identity, whereas the degree of homology between members of different families ranges from 50 to 70%.

Figure 3 shows the V_H gene family usage by our panels of hybridomas generated from neonatal spleen cells from conventional mice (indicated as CV-NEO) and from adult spleen cells from GF-CD mice (indicated as GF-CD). This V_H gene family usage is compared to the size and position of the V_H gene families analysed. In both hybridoma panels the PC7183 V_H gene family is preferentially used, and thus V_H gene usage is biased towards C_H proximal V_H segments. Data from the literature suggest that the V_H genes coding for the multireactive and autoreactive antibodies belong predominantly to the PC7183 family (*Painter* et al., 1986).

In hybridoma panels from adult spleen cells from conventional mice, V_H usage is primarily dependent on V_H family size instead of family position (*Dildrop* et al., 1985; *Schulze* and *Kelsoe*, 1987). Apparently, V_H family usage in conventional mice normalises during ontogeny.

If exogenous antigenic stimulation is avoided during the lifetime, clonal selection will hardly occur. Therefore, the usage of V_H genes by adult GF-CD mice will be more or less comparable to that of the neonatal B-cell pool. In adult GF-CD mice, however, besides a higher percentage of B-cells expressing the PC7183 V_H gene family, there is also a higher percentage of B-cells expressing the J558 family compared to neonatal B-cells. This suggests that normalisation has taken place to some extent in adult GF-CD mice. This could be caused by some endogenous antigenic stimulation, e.g. from idiotypes and other autoantigens, as suggested by the rise of the number of background IgG- and IgA-secreting cells in maturing GF-CD mice (*Hooijkaas* et al., 1984; *Bos* et al., 1987).

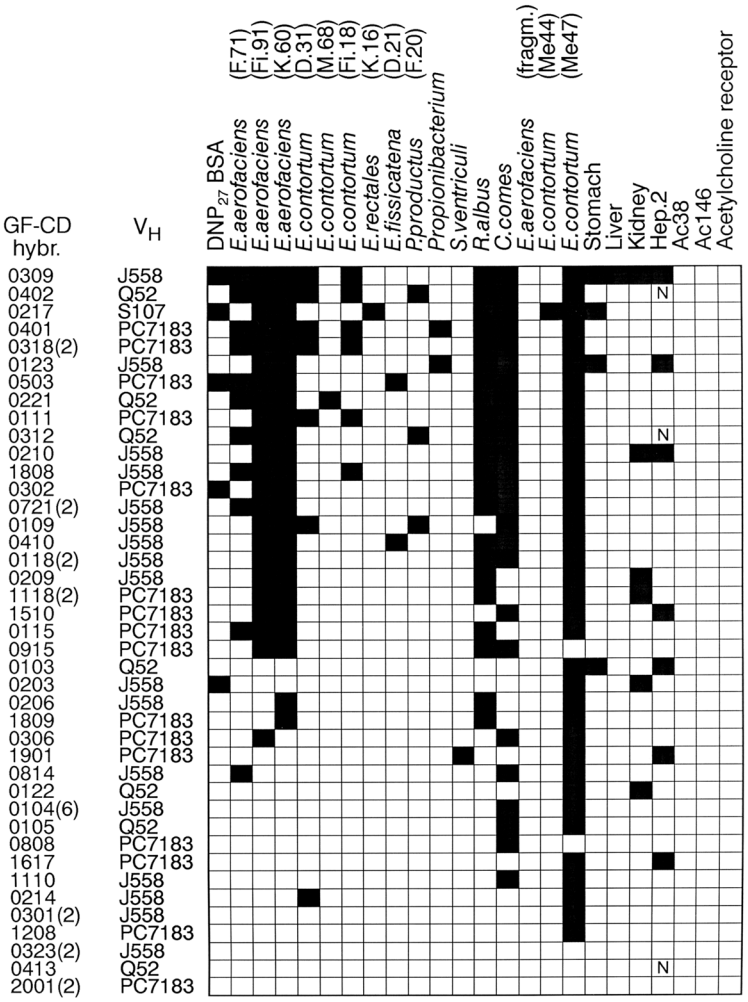

Figure 2: Reactivity pattern and V_H gene usage of a panel of hybridomas from adult "antigen-free" BALB/c mice. Closed squares represent positive reactions; open squares represent negative reactions; N = not done. Data from: *Bos* et al., 1989.

FACTORS INFLUENCING AUTOIMMUNITY

Both organism inherent factors (genetic factors etc.) and environmental factors influence the development and severity of autoimmunity.

The involvement of genetic factors is apparent from the observation that almost all autoimmune diseases show a preferential association to certain HLA alleles, especially HLA-DR2, -DR3, -DR4 and -B8 (*Nepom*, 1989). Immunodeficiencies that are genetically determined are often associated with autoimmune reactions. It is unclear, however, whether immunodeficiency directly influences autoimmunity, or that the autoimmunity is due to chronic infections, which more often occur in immunodeficiency (*Waldmann*, 1988). Also age and hormonal factors, particularly sex hormones, play a role in autoimmunity (*Wood* and *Badley*, 1986). Although many autoimmune

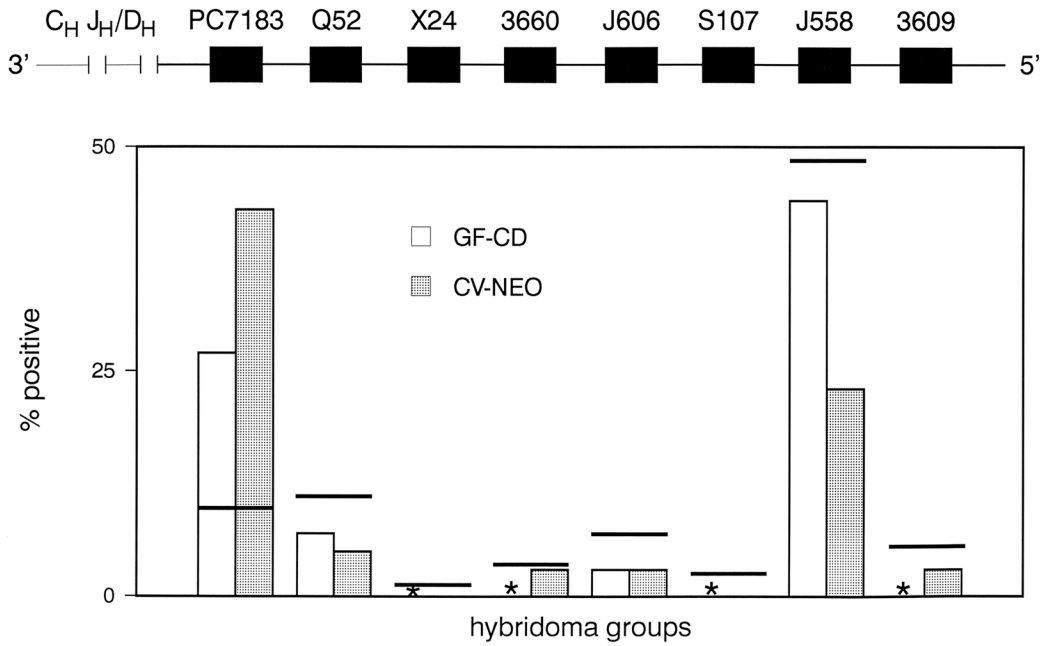

Figure 3: V_H gene family usage in GF-CD and CV-NEO hybridomas compared to the size and position of the V_H gene families. GF-CD (59) and CV-NEO (65) hybridomas were tested for V_H gene family usage by hybridisation of purified RNA to the different probes. Eleven GF-CD and 13 CV-NEO hybridomas could not be assigned to one V_H gene family. The horizontal line within each V_H gene family represents the expected percentage according to the size of that V_H gene family. The chromosomal order of the V_H gene families is shown at the top of the figures. * = not done. Data from: *Bos* and *Meeuwsen*, 1989.

diseases have an age-related peak incidence and the incidence of autoantibody production increases with increasing age (*Hawkins* et al., 1979), autoimmune diseases in general are not clearly related to older age groups (*Wood* and *Badley*, 1986).

As far as environmental factors are concerned, several drugs can induce autoimmune reactions. These are often asymptomatic and/or disappear after discontinuation of exposure. Also food, dust and other agents may cause autoimmune symptoms. Many infections by viruses, bacteria, fungi and parasites cause temporary autoimmune symptoms, particularly the production of rheumatoid factor and anti-nuclear antibodies. Usually these symptoms are reversible after eradication of the infectious agent, but tissue damage as the result of excessive immune reactions is not always reversible (*Wood* and *Badley*, 1986).

Evidence that infectious agents may be a major cause of autoimmune disease is accumulating (Table 4). In fact, many so-called autoimmune diseases may turn out to be the consequence of an infectious disease, with which the infected organism cannot cope with appropriately because of some selective immune response defect.

Table 4: Infectious agents suggested as inducers of autoimmune diseases

Rheumatoid arthritis	EB-virus related agents, Mycobacteria
Insulin-dependent diabetes mellitus	Coxsackie B virus
Multiple sclerosis	Defective measles virus
Sclerosing panencephalitis	C-type RNA virus
Sjögren's syndrome	A-type virus
Rheumatic fever	Group A streptococci
Ankylosing spondylitis	Klebsiella
Reiter's disease	Shigellae

POSSIBLE MECHANISMS OF POST-INFECTION AUTOIMMUNITY

Several mechanisms have been proposed for post-infection autoimmunity. These include: (a) change in endogenous antigen (*Bottazo* et al., 1986); (b) disturbance of the host immune response (*Marcos* et al., 1986); (c) molecular mimicry; and (d) somatic diversification of antibodies to environmental antigens (Table 5). From these, we shall discuss here the latter two.

Molecular mimicry between a microorganism and a host constituent

Research over the last few years has established that several microbial agents share determinants with human and animal proteins. This is called "molecular mimicry". An immune response mounted by the host against a specific determinant of an infecting agent may cross-react with the mimicked host-sequence, leading to an autoimmune reaction and, in some cases, tissue injury and disease. Evidence for the hypothesis that molecular mimicry can cause autoimmune disease in man comes from studies on the pathogeneses of ankylosing spondylitis and coeliac disease. *Schwimmbeck* et al. (1987) showed amino acid homology and immunological cross-reactivity between *Klebsiella pneumoniae* nitrogenase and the HLA-B27 variable domain. Similarly, *Kagnoff* et al. (1984) showed such similarities between Adenovirus 12E1B and protein A-gliadin, a dietary component of wheat gluten (Table 6). Such molecular mimicry between infectious agents and host sequences may frequently occur. However, unless the homology and subsequent immunological cross-reactivity involve a host protein that can precipitate disease, the autoimmune response is unlikely to lead to autoimmune disease (*Oldstone*, 1987).

Molecular mimicry between an antibody to a microorganism and a host constituent

The idiotypic network model of *Niels K. Jerne* (1974) implies that the variable regions of an antibody to a particular antigen can induce the production of complementary and interacting second order antibodies, the so-called anti-idiotypic antibodies. The idiotypic interconnection between first and second order antibodies and the B-cell subsets producing these antibodies is thought to regulate the immune response to the original antigen. Anti-idiotype antibodies induced as second order antibodies to a microorganism may functionally be autoantibodies (*Plotz*, 1983). That such autoantibodies may be relevant to autoimmune disease in man is suggested

Table 5: Possible mechanisms of post infection autoimmunity

Change in endogenous antigen
- tissue necrosis with release of intracellular antigen
- new antigenic determinants due to insertion of viral epitopes in cell membrane
- aberrant HLA class II expression
Disturbance of host immune response
- release of cytokines
- lymphotropic viruses
- microbial polyclonal activators (LPS, EBV, tuberculin, *B. pertussis*)
Molecular mimicry, i.e. sharing antigenic determinants between
- microorganism and a host constituent
- antibody against microorganism and a host constituent
Somatic diversification of antibodies to environmental antigens converting them into pathogenetic autoantibodies

by studies of *Dwyer* et al. (1986). They showed extensive idiotypic connectivity between antibodies against the acetylcholine receptor (AChR) and antibodies against α-1,3-dextran. Furthermore, they showed that 15% of patients with myasthenia gravis, which is caused by autoantibodies against the AChR, have serum antibodies against α-1,3-dextran. Control sera were negative for these antibodies. Certain of these anti-α-1,3-dextran antibodies were found to bind to anti-AChR antibodies via idiotypic interactions. As the α-1,3-dextran determinant is present on common opportunistic pathogens like *Enterobacter cloaca* and *Serratia liquefaciens*, these data suggest that myasthenia gravis may arise as the consequence of molecular mimicry between anti-α-1,3-dextran antibodies and the AChR. Naturally, susceptible individuals will have particular characteristics such as appropriate immune response genes (HLA-D), Ig-genes and/or T-cell abnormalities (*Dwyer*, 1988).

Somatic diversification of antibodies to environmental antigens converting them into pathogenetic autoantibodies

The antibody specificity repertoire is not only dependent on germline V-D-J gene rearrangement, deletion and other somatic diversification during B-cell differentiation, but also on somatic diversification of already expressed heavy and light chain variable regions. The

Table 6: Sequence similarities between microbial proteins and human host proteins

Disease	Protein	Residue	Sequence	Reference
Ankylosing spondylitis	*K. pneumoniae* nitrogenase	188	SRQTDREDE	*Schwimmbeck* et al., 1987
	HLA-B27	70	KAQTDREDL	
Coeliac disease	Adenovirus 12E1B	384	LRRGMFRPSQCN	*Kagnoff* et al., 1984
	Wheat gluten A-gliadin	208	LGQGSFRPSQQN	

latter form of diversification includes somatic point mutation, gene conversion and V region replacement (*Perlmutter*, 1989). Each of these events involved in the generation of antibody diversity may play a role in the production of pathogenetic autoantibodies.

Scharff and co-workers have shown that somatic point mutation in an Ig can convert an antibody reacting to an exogenous antigen into a potentially pathogenetic autoantibody (*Behar* et al., 1988). They isolated spontaneous mutants from the *in vitro* growing murine S107 myeloma cell line. The antibodies produced by this cell line bind to a number of bacterial polysaccharides found in the intestine of normal mice (*Potter*, 1972) and react specifically with phosphocholine which is present on these bacterial polysaccharides. Scharff and co-workers especially paid attention to mutants which had lost the ability to bind phosphocholine. One of these mutants was shown to have a single amino acid substitution of an alanine for glutamic acid at residue 35 in the first hypervariable region. This amino acid substitution was the result of a single base change that arose through somatic point mutation (*Giusti* et al., 1987). Most interestingly, this mutant not only had lost the ability to bind phosphocholine, but had acquired the ability to bind double-stranded DNA, phosphorylated proteins and phospholipids and therefore resembled autoantibodies found in lupus-like syndromes (*Schattner*, 1987; *Diamond* and *Scharff*, 1984). Thus, somatic mutation can convert a protective antibody into a potentially harmful autoantibody.

CONCLUSION

Normal adult humans and mice, and even new-borns, have B-lymphocytes which secrete antibodies recognising a variety of self-antigens. These "natural autoantibodies" usually are of the IgM class, of low affinity and a broad specificity (*Schattner*, 1987; *Holmberg* et al., 1986a). They seem not to account for autoimmune disease. In contrast, natural autoantibodies have been suggested to prevent autoimmune disease (*Cohen* and *Cooke*, 1986). Pathogenetic autoantibodies, on the other hand, mostly are of the IgG class, have a high affinity to the recognised antigen and have a narrow specificity (*Bottazzo* et al., 1986). In some autoimmune diseases the serum level of these autoantibodies correlate to disease activity, in other not (*Schattner*, 1987; *Holborow*, 1986), indicating that still much has to be learned about the pathophysiology of autoimmune disease.

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