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Anti-HIV and Anti-Anti-MHC Antibodies in Alloimmune and Autoimmune Mice

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Alloimmune mice (mice that have been exposed to cells from another murine strain) were shown to make antibodies against gp120 and p24 of human immunodeficiency virus (HIV), and mice of the autoimmune strains MRL-*lpr/lpr* and MRL-*+/+* made antibodies against gp120. This is surprising because the mice were not exposed to HIV. Furthermore, anti-anti-MHC antibodies (molecules that have shapes similar to those of major histocompatibility complex molecules) were detected in both alloimmune sera and MRL mice. These results are discussed in the context of a possible role for allogeneic stimuli in the pathogenesis of acquired immunodeficiency syndrome, as suggested by an idiotypic network model.

THE FUNCTIONAL DISRUPTION OF the immune system in acquired immunodeficiency syndrome (AIDS) is incompletely understood and may involve autoimmunity (1). Shearer suggested a possible role for allogeneic cells (cells from another individual) in AIDS pathogenesis on the basis of similarities between AIDS and graft-versus-host disease (2). Exposure to allogeneic cells can occur when individuals are exposed to foreign lymphocytes in blood transfusions or ejaculates. Ziegler and Stites (3) and Andrieu and co-workers (4) formulated an idiotypic network model for HIV in which it is suggested that gp120 of HIV may cross-react with class II MHC molecules, and an anti-idiotypic component of an immune response to gp120 could cross-react with the CD4 molecules on helper T cells. Such ideas are supported by the findings that large quantities of antigen-mimicking antibodies can be produced in ordinary immune responses (5), and many antibodies have both anti-idiotypic and antigen-specific activity (6). We formulated an autoimmunity model of AIDS pathogenesis that involves two immune responses, namely the immune response to HIV and an immune response to allogeneic stimuli (7). The two responses include components that are directed against each other, and these responses are postulated to synergize in a

way that causes the collapse of the immune system.

Two separable classes of specific antibodies can be detected in alloimmune sera, namely anti-foreign and anti-anti-self (8). Anti-anti-self antibodies are made in response to foreign idiotypes that recognize self MHC and may have MHC-image (MI) activity, as determined by their ability to completely inhibit cytotoxicity mediated by polyclonal alloantisera (8). This component of immunity to allogeneic lymphocytes may be related to immunity induced to HIV, because the envelope protein of HIV (gp160) has a shape that may be partly MI. One of its components (gp120) binds to CD4 at a site that overlaps with the site where CD4 interacts with class II MHC (9, 10), and there is also cross-reactivity between the other component (gp41) and class

II MHC (11). Furthermore, sequence similarities have been observed between gp120 and class II MHC (12) and between HIV-1 Nef and class II MHC (13). Because HIV components may have shapes that are partly MI, it is plausible that HIV provokes an immune response that includes an anti-class II MI (anti-MI) component. MI and anti-MI responses are by definition directed against each other, so MI and anti-MI lymphocytes could be stimulated by each other. Hence, an MI response to allogeneic cells could synergize with an anti-MI response to HIV (7).

Helper T cells have variable (V) regions that are selected to be weakly anti-self class II MHC. We have described an idiotypic network model in which suppressor T cells recognize and regulate helper T cells by means of their V regions; these T cells have receptors with class II MI determinants (14). The MI response to allogeneic cells and the anti-MI response to HIV could also be directed against anti-MHC idiotypic determinants of helper T cells and MI idiotypic determinants on suppressor T cells, respectively, and this dual attack could lead to the eventual collapse of the normal self-stabilizing system.

We considered the possibility that the image of MHC might be conserved across species, even though CD4, which has complementarity to class II MHC is not conserved between mice and humans with respect to its ability to bind gp120 of HIV. HIV gp120 binds to human CD4 but not mouse CD4. If the MI is at least partly conserved, alloimmune mice could make anti-MI antibodies that react with gp120. We raised alloimmune sera in pairs of strains of mice by repeated reciprocal immunizations with lymphoid cells. In agreement with previous work (8), the alloimmune sera were found to contain MI antibodies (15). These sera also contain antibodies to gp120 and p24 (Fig. 1, A and B). Similar results were obtained with all eight hyperimmune

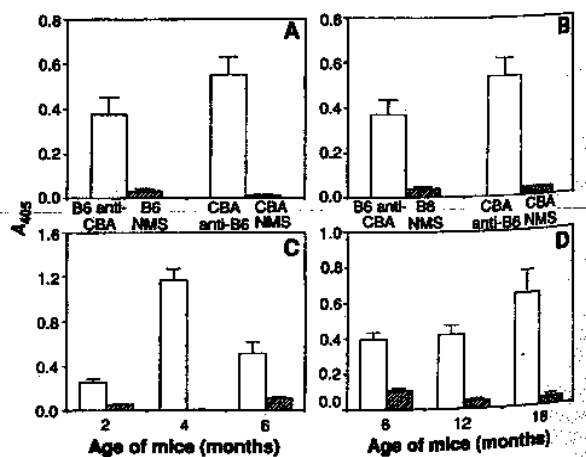


Fig. 1. Antibodies to gp120 (A) and p24 (B) were detected in B6 anti-CBA and CBA anti-B6 alloantisera, but not in normal (NMS) CBA or B6 sera. Antibodies to gp120 were also detected in the sera of MRL-*lpr/lpr* mice (C) (white bars) and in MRL-*+/+* mice (D) (white bars) but not in age-matched CBA controls (hatched bars) (17). A_{405} , absorbance at 405 nm.

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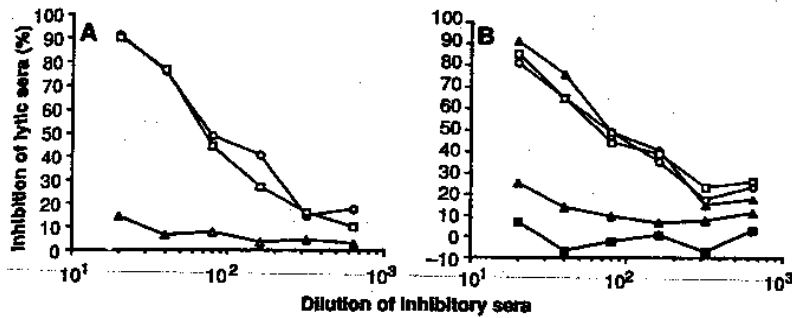


Fig. 2. Anti-anti-self MHC antibodies (putative MI) in MRL-*lpr/lpr* sera inhibit anti-H-2* antibody plus complement-mediated cytotoxicity. (A) Sera from 2-month-old MRL-*lpr/lpr* mice specifically inhibit killing of CBA lymph node cells by BALB/c anti-CBA sera (O) and killing of B10.BR cells by B10.D2 anti-B10.BR sera (anti-H-2*) (□). The same sera do not inhibit killing of BALB/c cells by CBA anti-BALB/c sera (Δ). (B) Inhibition of BALB/c anti-CBA cytotoxicity by sera from 2-month-old MRL-*lpr/lpr* mice both before (Δ) and after absorption of the sera with normal CBA (□) or BALB/c (○) immunoglobulin indicates that the inhibition is not caused by rheumatoid factor. Sera from 1-month-old MRL-*lpr/lpr* mice (■) and sera from 2-month-old MRL-*lpr/lpr* mice absorbed with rabbit anti-mouse Ig (▲) are not inhibitory (19).

mouse alloantisera we tested, namely C57BL/6 (B6) anti-CBA, CBA anti-B6, BALB/c anti-B6, B6 anti-BALB/c, C3H anti-C3H.SW, C3H.SW anti-C3H, B10 anti-B10.BR, and B10.BR anti-B10 (15).

The detection of antibodies to p24 in alloimmune sera was completely unexpected. Taken together with the similarity between gp120 and class II MHC (9, 12), the cross-reaction of gp41 and class II (11), and the homology between Nef and class II MHC (13), this observation suggests that there has been evolutionary pressure for HIV antigens to be MI. The presence of antibodies to p24 and gp120 in alloantisera supports the idea that there can be synergy between allogeneic stimulation and stimulation by HIV.

MRL-*lpr/lpr* mice die at about age 6 months from a progressive autoimmune disease and are considered to be a model of

the human disease systemic lupus erythematosus (SLE). MRL-+/+ mice suffer from a milder form of the disease and live to about 18 months. Kaye has listed 31 clinical and serological features that are common to SLE and HIV infection (16). It is thus plausible that the mechanisms of pathogenesis are related, even though SLE occurs spontaneously and AIDS is provoked by HIV. We tested MRL-*lpr/lpr* and MRL-+/+ mice for the presence of MI and anti-HIV antibodies. An enzyme-linked immunosorbent assay (ELISA) experiment (17) indicated that these mice spontaneously make anti-gp120 antibodies, which we interpret as anti-MI (Fig. 1, C and D). In a titration experiment with pooled sera, we were unable to detect antibodies to p24 in MRL-*lpr/lpr* or MRL-+/+ mice (15). This latter result is a negative control, consistent with the idea that anti-gp120 activity is due to specific

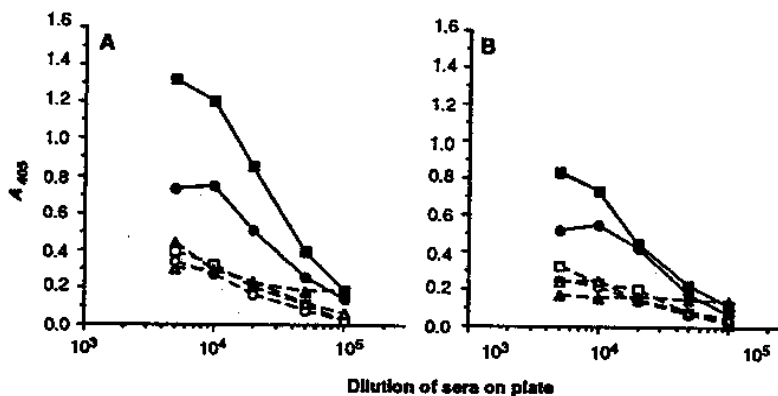


Fig. 3. Anti-anti-MHC antibodies in the sera of MRL-*lpr/lpr* autoimmune mice detected by ELISA with monoclonal anti-H-2* antibodies. Anti-anti-class I and anti-anti-class II antibodies are seen in 2-month-old MRL-*lpr/lpr* (A) and 12-month-old MRL-+/+ (B) mice. MRL-*lpr/lpr* and MRL-+/+ sera showed reactivity to anti-I-A* (■) and anti-H-2K^bD^b (●) monoclonal antibodies, but not to anti-I-A* (□), anti-H-2K^bD^b (○), anti-V_{B8} (▲), and anti-β-2-6-linked-fructosan (Δ) IgG2a monoclonal antibodies (20).

antibodies. The specificity of these antibodies was confirmed in an inhibition experiment (18). Free gp120 (2.5 μg/ml) added to the ELISA assay gave 82.8 ± 0.7% (mean ± SEM, n = 5) inhibition of the signal for antibodies from MRL-*lpr/lpr* mice binding to gp120, whereas the same concentration of p24 gave 0.5 ± 6.4% (n = 5) inhibition. Similar results were obtained for the antibodies to gp120 in MRL-+/+ mice, namely 78.5 ± 6.5% (n = 5) inhibition by gp120, compared with 1.8 ± 5.8% (n = 5) inhibition by the same amount of p24.

Anti-anti-self (putative MI) antibodies were also detected in the sera of MRL mice. Sera from 2-month-old MRL-*lpr/lpr* mice (H-2*) inhibited BALB/c anti-CBA and B10 anti-B10.BR (anti-H-2*) but not CBA anti-BALB/c cytotoxicity (Fig. 2A). We concluded that the inhibiting activity was caused by an antibody because it was absorbed out with rabbit anti-mouse immunoglobulin (Ig) (Fig. 2B). Furthermore, the inhibiting activity was not caused by rheumatoid factor; the activity is specific for anti-H-2* (Figs. 2A and 3) and is not absorbed out by BALB/c Ig or CBA Ig (Fig. 2B).

Anti-anti-self MHC antibodies were also detected in MRL-*lpr/lpr* and MRL-+/+ sera by means of an ELISA assay with monoclonal antibodies to H-2 (Fig. 3). Reactivity was detected with the V regions of both anti-I-A* and anti-H-2K^bD^b monoclonal antibodies with anti-I-A^b, anti-H-2K^bD^b, anti-V_{B8}, and anti-β-2-6-linked-fructosan as negative controls. All of these monoclonals are IgG2a antibodies. Specific reactivity at a dilution of greater than 1 in 10⁴ was seen against both anti-class I and anti-class II monoclonals directed against the *k* haplotype, with anti-anti-class II reactivity stronger than anti-anti-class I reactivity. We tested five anti-H-2* monoclonal antibodies in this assay and observed anti-anti-H-2* reactivity with four of them. The one that had no reactivity was an anti-I-E* monoclonal (15).

We conclude that both anti-anti-MHC (presumably MI) and anti-gp120 (that can be interpreted as being anti-MI) are found in both alloimmunity and autoimmunity. The inherent instability in the immune system of MRL-*lpr/lpr* and MRL-+/+ mice that leads to autoimmunity thus occurs together with presumptive MI and anti-MI antibodies. This supports an MI-anti-MI model of pathogenesis for these autoimmune mice. The presence of both kinds of antibodies in alloimmune mice also supports the idea of synergy between immune response to allogeneic cells and HIV antigenic stimuli, as postulated (7).

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10. One question that might be raised is whether there is overlap between the CD4 binding site on class II MHC and the T cell receptor binding site on class II MHC. The experiments that localize the interaction of the T cell receptor with class II MHC exclusively to the neighborhood of the cleft on the MHC molecules are the same ones that are concerned with the interaction of T cell receptors with peptides in the clefts. We have questioned the conventional absolute interpretation of such data [G. W. Hoffmann and M. D. Grant, *Lect. Notes Biomath.* 83, 386 (1989)]. The data typically come from experiments in which the T cells have been primed to an antigen and therefore reflect the properties of a part of the repertoire that are biased by the priming process. Different antigen fragments survive for different lengths of time. The fragments that are broken down most slowly are presumably stimulatory for the longest time, and fragments that happen to fit into a cleft of the MHC molecules will be protected from proteolytic degradation. One might expect those fragments to persist in stimulating a subset of T cells that then become the dominant population specific for a particular antigen + MHC. Therefore, the specificity repertoire of primed T cells is a reflection of the selection process for antigen fragments, and it may be a mistake to extrapolate from that repertoire to the naive T cell repertoire. Naive helper T cells may include clones that interact with various exposed parts of MHC class II, including the CD4 binding site, and not just those residues near the cleft.
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17. ELISA plates coated with 20 ng per well of recombinant gp120 or recombinant p24 (MicroGeneSys, West Haven, CT) and blocked with 5% casein were incubated with sera diluted 1 in 100 with phosphate-buffered saline (PBS)-Tween 20 plus 1% casein. The bound antibodies were detected with biotinylated-goat anti-mouse IgG (Bethesda Research Laboratories-Gibco, Burlington, Canada) and avidin conjugated to alkaline phosphatase (Calbiochem). The plates were incubated with the substrate for 3 hours (Fig. 1, A and B) or 1 hour (Fig. 1, C and D). The numbers of mice for Fig. 1, A and B (independent samples), were five B6 (normal), five B6 (immunized), six CBA (normal), and six CBA (immunized); and for Fig. 1, C and D, the numbers were ten for each MRL group and eight for each CBA group.
18. The ELISA was performed as described in (17), except that the sera were diluted 1 in 200 (final concentration) and were added together with the inhibitor. The results given are the mean inhibition \pm SEM for five each of the mouse strains MRL-*Apr/pr* (age 4 months) and MRL-*+/+* (age 12 months) (clone 16-1-2N), anti-I-A* (clone AF6.120.1.2), and anti-H-2K^bD^b (clone 12-2-2S) were obtained from the American Type Culture Collection. Anti- β -2-6-linked-fructosan (clone UPC.10) was purchased from Sigma. Anti-V_{H8} (clone F23.1) was a gift from H.-S. Teh, University of British Columbia, Vancouver, BC. Pooled sera from 15 mice (MRL-*Apr/pr*) and 10 mice (MRL-*+/+*) were used in this experiment. Similar results were obtained in a separate single-point experiment in which we tested sera from individual mice with 8 to 10 mice per group. The anti-I-A* and anti-H-2K^bD^b monoclonal antibodies do not react significantly with normal CBA sera (15).
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Specific DNA Binding by c-Myb: Evidence for a Double Helix-Turn-Helix-Related Motif

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The c-Myb protein is a sequence-specific DNA binding protein that activates transcription in hematopoietic cells. Three imperfect repeats (R₁, R₂, and R₃) that contain regularly spaced tryptophan residues form the DNA binding domain of c-Myb. A fragment of c-Myb that contained the R₂ and R₃ regions bound specifically to a DNA sequence recognized by c-Myb plus ten additional base pairs at the 3' end of the element. The R₂R₃ fragment was predicted to contain two consecutive helix-turn-helix (HTH) motifs with unconventional turns. Mutagenesis of amino acids in R₂R₃ at positions that correspond to DNA-contacting amino acids in other HTH-containing proteins abolished specific DNA binding without affecting nonspecific DNA interactions.

THE C-MYB NUCLEAR ONCOPROTEIN is a transcriptional activator whose expression is linked to the differentiation state of hematopoietic cells (1, 2). The c-Myb protein functions in expression of *mim-1*, *c-myc*, *cdc2*, and the gene that encodes DNA polymerase α (3). It also activates transcription from the human immunodeficiency virus-1 long terminal repeat (4). Oncogenic activation of *c-myb* can occur when truncated versions of c-Myb are expressed that give rise to versions that lack either an NH₂-terminal phosphorylation site that regulates specific DNA binding (5) or a COOH-terminal trans-repressor domain (1). In addition, point mutations in the DNA binding domain can impose alternative differentiation phenotypes on transformed myeloid cells (6). The DNA binding domain is located near the NH₂-terminus and is composed of three highly conserved,

imperfect 51- or 52-residue repeats (designated R₁, R₂, and R₃); only R₂ and R₃ are required for sequence-specific DNA binding (7, 8). Each repeat contains three regularly spaced tryptophans that are important for maintaining an active DNA binding structure (9, 10). In order to examine the minimal DNA binding domain, we engineered a 312-bp region of chicken *c-myb* (11) that encoded the R₂R₃ domain by the polymerase chain reaction (PCR) for expression in *Escherichia coli* (12). The R₂R₃ recombinant polypeptide was purified to near homogeneity (13) for use in the studies.

We used the electrophoretic mobility shift assay (14) to monitor DNA binding to an oligonucleotide that contained two Myb recognition elements (2xMRE-probe). Two complexes (C1 and C2) were observed (Fig. 1A) in a proportion that was dependent on the protein-to-DNA ratio. When increasing amounts of protein were added, complex C1 was formed first, followed by C2, which was the predominant complex at high protein-to-DNA ratios. Competition with specific and nonspecific oligonucleotides showed that both complexes are specific (15). These results demonstrate that R₂R₃ is sufficient

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