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The importance of the back-signal from T cells into antigen-presenting cells in determining susceptibility to parasites

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SUMMARY

It has long been known that certain MHC class II genes can dominantly suppress immune responses and so increase susceptibility to parasite infections, but the mechanism has been unclear. Recent work has revealed one way in which this form of suppression may operate, through gating by MHC class II molecules of the back-signal from activated T cells into macrophages. The two known suppressive genes of the mouse are expressed in macrophages more extensively than are other class II genes. This is asscociated with suppression of IL-4 production resulting, we infer, from overproduction in the macrophages of IL-12, the counter-cytokine to IL-4. The lack of IL-4 may itself be immunosuppressive, even for Th2 responses, and excess IL-12 can overinduce the antiproliferative cytokine IFN-gamma. Although this mechanism requires further substantiation, we believe that it offers a reasonable answer to an old conundrum.

The ability of certain class II MHC genes to suppress antiparasite immune responses began to attract attention during the 1980s. Expression in the mouse of *H2E* was found to reduce the protective response to nematode infection (Wassom *et al*. 1987) and to visceral leishmaniasis (Blackwell & Roberts 1987), although the effect is not always obtained (Behnke & Wahid 1991). In man, expression of certain HLA.DQ alleles reduced the response to schistosome antigens (Hirayama *et al*. 1987). It has been suggested that genes responsible for this type of activity might be retained in natural populations by the selective advantage conferred by reducing damage from inflammation (Mitchison & Oliveira 1986). However, little progress was made, mainly because the mechanism of suppression was not understood. That problem has now been solved, in part at least, as summarized here. The solution turns on polymorphism in the promoters of the class II MHC genes in question.

We were led to investigate this type of polymorphism by our longstanding interest in the protective and suppressive function of MHC class II genes (Silver & Lane 1975). The first sign of an effect unlikely to be mediated by the classical exon function of determinant selection was the discovery that expression of the *H2E* gene suppressed the immune response not only to foreign lactic dehydrogenase (Nagy *et al*. 1981), but also to many other antigens (Oliveira & Mitchison 1989). Particularly relevant was the finding that the response to a whole range of structurally linked antigens could be suppressed at the same time, such as that to an assembly of non-MHC alloantigens presented on the same cell (Krzych *et al*. 1989); much the same must have occurred in the suppression of the antiparasite responses mentioned above. Subsequently the *b* allele at *H2A* was found to have a similar but weaker effect, which operates additively with that of *H2E* (Hesse *et al*. 1996). This line of research has now moved on to the prevention and cure of autoimmune disease in mouse models (Hirose *et al*. 1994; Gonzalez-Gay *et al*. 1994; Mitchison & Brunner 1995). It has obvious relevance to human immunological diseases, where this genetic approach to cure makes a welcome change from the dauntingly difficult task of determining the causation of these diseases (Mitchison 1992).

'Suppression' is an appropriate term to use, as these genes are effective in a single dose and must therefore play an active role, rather than one of passive inactivation. Their activity in this respect represents no more than a bias, as they can also function as normal positive immune response genes. There is evidence of similar effects in man, particularly among DQ and DR2, 5 and 7 haplotypes; and an allele that is otherwise associated with protection may cease to be protective in a disease for which it is a susceptibility factor, as in the case of juvenile chronic arthritis and perhaps also in multiple sclerosis (Guardiola *et al*. 1996).

Some MHC class II promoters are highly polymorphic, to an extent that indicates that they must be under selective pressure (Guardiola *et al*. 1996). In contrast, MHC class I promoters are relatively constant (Cereb & Yang 1994; Yao *et al*. 1995). Especially telling is the finding that sequence variation

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between allelic promoters in the mouse is actually greater within (and immediately adjacent to) the X and Y boxes than in the intervening sequences (Janitz *et al*. 1997). These boxes are where transcription factors are known to bind, and where sequence is conserved over long evolutionary distance (Benoist & Mathis 1990; Sultmann *et al*. 1993); indeed this conservation was one of their original defining features. The situation is slightly different in man, where more alleles have been sequenced, and variation within the X and Y boxes was again found to be high, but not quite as much so as in the intervening sequences (Louis *et al*. 1993). For both man and mouse these generalizations are provisional, as there are many more promoters to sequence; it would be of interest to learn about promoter diversity within the mouse genus, for example. What seems clear is that the functionally active part of these promoters must be under selective pressure to diversify, presumably reflecting increased fitness of heterozygotes.

Analysis of cytokine gene expression provides a clue to the mode of action of these suppressive/protective genes. A survey of the major T-cell cytokines revealed that the most conspicuous effect of the $H2A^b$ allele is to inhibit the burst of IL-4 transcription that occurs soon after immunization in a suppressible immune response; *in vivo* treatment with anti-IL-4 monoclonal antibody mimics this effect, provided that the antibody is given immediately upon immunization (Brunner *et al*. 1995; Hesse *et al*. 1996). As the suppressive allele is known to play a positive role, a reasonable inference is that it elicits a counter-cytokine to IL-4.

Our most recent finding is that the suppressive genes $H2A^b$ and *H2E* are both expressed more extensively in activated macrophages than are the neutral alleles $H2A^d$, $H2A^k$ and $H2A^q$. This parallels an earlier finding that promoter–reporter gene constructs transfected into a macrophage cell line yield a higher signal when made with the $H2A^b$ promoter. The increase results from a single A→G substitution at the 3′ end of the X box in the $H2A^b$ promoter, which is reversible by site-specific mutagenesis (Janitz *et al*. 1997).

Recent discoveries concerning IL-12 offer a solution to this puzzle, shown in figure 1. IL-12 is the principal counter-cytokine to IL-4 (Seder *et al*. 1996; Abbas *et al*. 1996). It is made mainly by activated macrophages and dendritic cells, in response to back-signals delivered by recently activated T cells. The ligand-pair that actually delivers the signal is CD40L on the T cell ligating to CD40 on the antigen-presenting cell (APC), but signalling is facilitated by ligation of MHC class II molecules on the APC to the T-cell receptor (Kato *et al*. 1995; Koch *et al*. 1996; Cella *et al*. 1996). It is reasonable to suppose that the level of expression of MHC class II gates the strength of this signal, so that increased expression leads to greater production of IL-12, which would in turn inhibit IL-4 production. One may also suppose, as shown in figure 1, that these interactions take place among T cells clustering around an APC (although not necessarily all at the same time). The IL-12-inducing T cell binds via a suppressive MHC class II molecule, and the target T cell, which would otherwise mediate a positive response, binds via a positively-inducing class II molecule. An interaction of this sort between two T cells, which occurs via a change induced in their common APC, has been termed 'epicrine' (T. Tada, personal communication), a useful term.

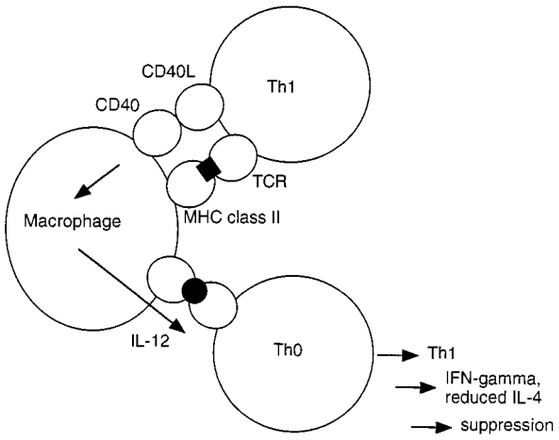

Figure 1. Epicrine interaction between a T cell recognizing a suppressive epitope (solid square) and one recognizing what would otherwise be a positive-response-inducing epitope (solid circle). The level of expression of the MHC class II molecule recognizing the suppressive epitope gates the back signal (delivered via CD 40L) into the macrophage; heightened expression causes higher IL-12 production. The IL-12 is envisaged as having a suppressive effect via IFN-gamma induction (Caspi *et al*. 1997) and reduced IL-4 levels.

The only surprising feature of this hypothesis is the suppressive role of IL-12, a molecule which on the basis of *in vitro* experiments is considered essential for development of a Th1 response (Abbas *et al*. 1996). Collagen-induced arthritis, known to be susceptible to the suppressive effect of $H2A^b$ and *H2E*, is mainly a Th1 disease like other organ-specific autoimmunities induced with complete Freund's adjuvant. However, there is something odd about these diseases. Although IL-12 given during the course of the disease has an exacerbating effect, when given at the time of induction it is protective (Hess *et al*. 1996; Caspi *et al*. 1997). This mirrors the timing seen with anti-IL-4 treatment referred to above. The protective effect appears to operate via induction of IFN-gamma, because knock-out of this gene exacerbates at least one of these diseases (Krakowski & Owens 1996) and blocks the protective effect of early treatment with IL-12 in another (Caspi *et al*. 1997). All in all, the picture derived from *in vitro* studies on TCR-transgenic T cells (Abbas *et al*. 1996) may be oversimplified. It seems likely that an important distinction applies *in vivo* between an initial proliferative phase of the immune response, in which IL-4 may be important as a growth factor for both Th1 and Th2 cells and IFN-gamma as an antiproliferative agent (Chiodetti & Schwartz 1995; Krakowski & Owens 1996), and a later phase in which the role of these cytokines in maintaining Th1/Th2 balance becomes more important; the role of IL-12 in the second phase is the subject of a recent review (Seder *et al*. 1996).

The scheme presented in figure 1 is unlikely to be the only mechanism by which differential expression of MHC class II genes influences the immune response. Suppressive MHC molecules have hardly begun to be studied in man, where the lack of suitable monoclonal antibodies is an obstacle. What is presented here is simply the first mechanism to be worked out in detail. It will no doubt be tested in future investigations, but there are many other possible mechanisms (Gonzalez-Gay *et al*. 1994; Guardiola *et al*. 1997).

To complete this presentation, a note about the association of promoter and exon variation. An extreme position would be that the polymorphism observed in promoters merely reflects—is dragged along by—the well-known polymorphism of exons. This seems increasingly unlikely in view of the arguments deployed above. However, it is unlikely that the two are entirely independent of one another. Very little is known about why particular MHC alleles are retained in natural populations, although it is generally assumed that this reflects the need to present the antigens of pathogens. Suppression of the response to pathogen antigens would also be appropriate under certain circumstances, particularly where chronic immunopathology would otherwise develop (Mitchison & Oliveira 1986); leprosy is a case in point, where an immunosuppressive, dominant, HLA-linked effect favours progress into the less life-threatening lepromatous form of the disease (van Eden *et al*. 1985), a development which is beneficial to both host and pathogen. In a scenario of this sort it is not difficult to imagine how a suppressive level of expression could come to associate, during the course of evolution, with the ability to present a particular set of epitopes.

The argument presented here is that an early burst of IL-12 production can suppress the immune response independent of (or at least in addition to) its Th1-inducing effect. If so, it clearly presents a way of subverting the immune response that is likely to have attracted the attention of parasites. It is true that mice respond to the injection of large doses of *Leishmania major* in the opposite way, by producing an early burst of IL-4 (Launois *et al*. 1995). In the light of the Blackwell & Roberts (1987) findings, the hypothesis advanced here makes the following sharp prediction: when administered by the route and in the dose used in the that earlier study, *L. donovani* should elicit less early IL-4 and more early IL-12 in mice that express *H2E* and $H2A^b$ than in control, non-expressing mice of the same genetic background (e.g. in B10.A(5R) versus B10.A(4R) or B10.Q). In summary, the possibility that parasite-mediated suppression may on occasion be detrimental to the host should not be neglected, and we should be alert to the danger that precocious induction of IL-12 may present.

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