basis for the genetic selection (duration of the loss of the righting reflex).

Our results indicate that a gene expression system can be used to define genetic differences in brain function. Expression studies in coordination with pharmacological genetic selection offer a promising strategy for the study of drug-receptor interactions.

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MHC-Linked Protection from Diabetes Dissociated from Clonal Deletion of T Cells

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The I-E molecule of the major histocompatibility complex (MHC) can prevent the spontaneous development of diabetes in nonobese diabetic (NOD) mice. The mechanism of this protection has been investigated by breeding wild-type and promoter-mutated E^k_α transgenes onto the NOD genetic background. Animals carrying the various mutated transgenes expressed I-E on different subsets of immunocompetent cells, and thus cells important for the I-E protective effect could be identified. Although the wild-type transgene prevented the infiltration of lymphocytes into pancreatic islets, none of the mutants did. However, all of the transgenes could mediate the intrathymic elimination of T cells bearing antigen receptors with variable regions that recognize I-E. Thus, the I-E molecule does not protect NOD mice from diabetes simply by inducing the deletion of self-reactive T cells.

THE NOD MOUSE PROVIDES A MODEL for studying the immunology of insulin-dependent diabetes mellitus (IDDM) in humans (*1*). The murine disease is similar to the human condition in several ways: (i) it is caused by specific T lymphocytes that invade and destroy the pancreatic islets (*2–4*); (ii) this can be alleviated or prevented by immunosuppressive reagents such as cyclosporin (*5*); and (iii) the disease, although under complex polygenic control, is influenced by a gene (or genes) in the major histocompatibility complex (MHC) (*6–9*).

The class II genes of the NOD MHC constitute a distinct haplotype: the I-A complex has a β chain of unusual sequence (*10*), and the I-E complex is absent because of a deletion in the α chain gene (*7*, *11*). The importance of MHC class II genes, in particular of the defective E_α locus, has been demonstrated by introducing an E^d_α transgene onto the NOD genetic background: this gene prevented lymphocyte infiltration into the pancreatic islets of 10-week-old mice (*12*). However, this result has not been accepted without question [see discussion in (*8*), for example]. Because only one transgenic line was used, the protection from insulitis could have resulted from the chromosomal integration site of the transgene, from special features of the transgene construct, or from peculiarities of the E_α allele employed. Several hypotheses have been suggested to explain the protection phenomenon (*4*, *12*). In one, diabetes in NOD mice was postulated to result from an autoimmune attack by T cells that carried the $V_\beta5$ variable region on their T cell antigen receptors (TCRs). Since T cells displaying $V_\beta5^+$ TCRs are negatively selected in the thymus of mice expressing the I-E molecule (*13*), the diabetogenic anti-islet clones would be deleted intrathymically when a wild-type E_α gene is introduced into NOD mice (*4*).

To verify the original observation and to assess where E_α must be expressed to protect from insulitis, we have crossed the NOD strain with a number of E^k_α transgenic lines. One of them, $E_\alpha16$, expresses the I-E

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complex on all cells that normally display class II molecules. In the other three lines, Sma 58, WED 21.16ΔX (ΔX), and WED 301.54ΔY (ΔY), expression is limited to particular compartments of the immune system (*14, 15*) (Fig. 1). Mice from each of the transgenic lines (carried on a C57BL/6 background) were mated with NOD mice to produce F_1 generations, which in turn were backcrossed with NOD mice (Fig. 2). This first backcross generation was doubly selected for the presence of the transgene and for homozygosity at the NOD MHC by evaluation of Southern blots of tail DNA. Several of the selected animals were again backcrossed with NOD mice to produce the experimental generation [N_3 according to standard nomenclature (*16*)]. N_3 females were evaluated for insulitis at 10 to 12 weeks of age by examining pancreas sections (*17*) and, in parallel, were typed for the presence or absence of the transgene. By comparing E_α^{k+} and E_α^{k-} littermates in this manner, we evaluated the effect of the E molecule on insulitis, independent of other segregating loci. (The ostensibly simpler approach of injecting the mutant transgenes directly into NOD embryos was not undertaken because the interesting "compartmentalized" expression patterns were obtained fortuitously; that is, they occurred in only one of many independent lines.)

Control animals lacking the transgene had an insulitis incidence of about 40%, a value consistent with the hypothesis that as many as six loci from C57BL/6 mice dominantly protect from diabetes (*8*), but at odds with another report (*12*). This complex genetic control was also manifest in the intensity of insulitis exhibited by different animals. There was a distinct bimodal distribution: about one-fifth of the animals had more than 30% of their islets clearly infiltrated (as did all inbred NOD^+ controls); the remaining four-fifths had fewer than 15% affected islets, and they were often only marginally infiltrated.

The wild-type E_α^k transgene from the $E_\alpha 16$ line bestowed almost complete protection from insulitis (Fig. 1; $P < 0.02$). The only animal with any sign of T cell infiltration had fewer affected islets with only mild invasion. The protection was not due to a simple delay in the kinetics of autoimmune attack, as NOD/$E_\alpha 16$ mice remained insulitis-free even at 6 months of age (*11*). The prevention of disease in NOD mice by an E_α^k transgene confirms the previous observation with an E_α^d transgene (*12*); together these data indicate that protection from insulitis is due to the E_α gene itself, and is not an artifact of transgene insertion or linkage. Recent results with a NOD/E_α line generated by injecting an E_α^d gene directly into NOD embryos also confirmed this observation (*18*).

In contrast, none of the promoter-mutated transgenes significantly protected from insulitis. The frequency and intensity of insulitis was similar for N_3 backcross mice carrying the *ΔX*, *ΔY*, or *Sma* transgenes and for their transgene-negative littermates. Since this result was rather surprising, we confirmed by microscopic analysis of the appropriate fluorescently labeled tissue sections that the mutant transgenes were expressed on the NOD background exactly as they are on the C57BL/6 background (*11*).

The *ΔX*, *ΔY*, and *Sma* transgenes can mediate the intrathymic deletion of I-E–reactive T cells, a fact demonstrated for a variety of TCR V_β regions (5, 6, 11, and 17a) on a variety of genetic backgrounds (for example, C57BL/6, SJL, and SJL × DBA/1) (*11, 14*). Thus, the lack of protection by these transgenes seems inconsistent with the hypothesis that I-E expression prevents insulitis by eliminating $V_\beta 5^+$ T cells (*4*). To verify this point we stained peripheral lymphocytes from the NOD-backcrossed transgenic mice with an anti-$V_\beta 5$ reagent and quantitated them by cytofluorimetry (Table 1). Three major points emerge. First, as in other strains (*13*), by far the majority of $V_\beta 5^+$ T cells in NOD mice occur in the $CD8^+$ compartment. The reason for such a pronounced skewing is not yet known. Second, little I-E–mediated negative selection occurs on the NOD background: there is only moderate deletion of $V_\beta 5$-bearing T cells in NOD/$E_\alpha 16$ transgenic mice, and essentially no deletion of T cells displaying $V_\beta 6$ or $V_\beta 11$. The extent of negative selection is influenced by background genes, in some cases *Mls* (*19*), and these loci seem not to be very conducive to negative selection in NOD mice. Third, all of the transgenes induced the same amount

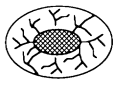
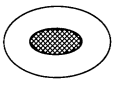
	Thymus	Periphery	Repertoire selection: Positive	Repertoire selection: Negative	Insulitis: Transgenics	Insulitis: Negative littermates
$E_\alpha 16$		B : 100% + Mϕ: +	+	+	1/18	10/26
ΔX		B : 100% + (heterogeneous) Mϕ: +	–	+	11/33	14/24
ΔY		B : 20-50%+ Mϕ: –	+	+	12/21	17/31
Sma		B : 4% + Mϕ: + (dull)	+	+	10/23	9/15

Fig. 1. E_α expression patterns and insulitis frequency in NOD/E_α transgenic lines. The expression (first two columns) and repertoire selection data are essentially as described previously (*14*), but have been confirmed on the NOD background (*11*). Thymus expression is illustrated in the first column: normal E_α expression on epithelial cells of the cortex is shown as a reticulum in the outer oval, no expression as a blank outer oval; normal E_α expression on epithelial cells, dendritic cells, and macrophages in the medulla is indicated as a stippled inner oval, no expression as a blank inner oval. Insulitis values denote the number of insulitis-positive mice with respect to the total number of mice conclusively scored (*17*).

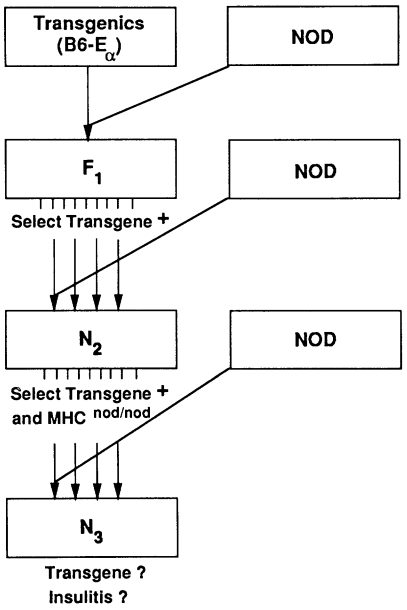


Fig. 2. Protocol for creation of the NOD/E_α lines. The NOD mice originated from a colony at the Department of Immunology, John P. Robarts Research Institute, London, Ontario, Canada. All of the females in our NOD colony develop insulitis.

Table 1. T cell antigen receptor V_β usage in NOD/E_α transgenic mice and E_α transgenics on SJL × DBA/1 and C57BL/6 genetic backgrounds. In individual mice, the percent of $CD4^+$ or $CD8^+$ cells that express a particular V_β is shown. TCR V_β usage was determined by cytofluorimetry (*22*). ND, not determined.

Transgene	NOD ($V_\beta5$)		SJL × DBA/1 ($V_\beta6$)	C57BL/6 ($V_\beta11$)
	$CD4^+$*	$CD8^+$	$CD4^+$	$CD4^+$
None	0.6, 0.9, 0.7, 0.7, 0.6, 0.7	7.6, 7.8, 6.0, 8.1, 8.9, 7.6	4.0, 4.4, 2.9	5.2, 4.6, 4.0, 4.4
$E_\alpha16$	0.1, 0.2, 0.4, 0.2, 0.0, 0.3	3.0, 3.1, 2.4	0.5, 0.4	0.2, 0.5
ΔX	0.0, 0.2	3.7, 3.9, 2.7	0.4	0.7, 0.8, 1.0
ΔY	0.2, 0.2	4.2, 3.7	0.2	0.4, 0.7
Sma	0.2, 0.2, 0.4, 0.5	2.5, 1.3, 2.2	ND	ND

*The background has been subtracted.

of clonal deletion: the *ΔX*, *ΔY*, and *Sma* transgenes, which did not protect from insulitis, eliminated $V_\beta5^+$ T cells similarly to the *$E_\alpha16$* transgene, which did protect. Clonal deletion of other TCR V_β regions by the same set of transgenes was more extensive on a C57BL/6 or (SJL × DBA/1) genetic background.

Thus, our data are clearly inconsistent with the hypothesis that the I-E molecule protects NOD mice from insulitis by mediating clonal deletion of $V_\beta5^+$ T cells. Other studies are also inconsistent: (i) none of the disease-provoking T cell clones isolated by other groups use $V_\beta5$, but instead, show rather heterogenous V_β usage (*20, 21*); (ii) immunohistological studies of islets in young NOD mice show that the infiltrating T cells use diverse V_β regions (*21*); (iii) treatment of NOD mice with anti-$V_\beta5$ antibody does not eliminate disease (*21*).

More generally, our results argue against any I-E–mediated clonal deletion event as we currently understand it. The results cannot be explained simply by segregation of other loci (such as, T cell receptor, Mls, and insulitis). We have scored E_α^+ and E_α^- littermates, so with large numbers from multiple crossings, we should only be measuring the influence of E_α. It is also unlikely that an unknown, and hence untested, T cell receptor ($V_\beta x$) is differentially deleted in wild-type E_α as compared with *ΔX*, *ΔY*, and *Sma* transgenic mice. We have examined V_β deletions using all available anti-V_β reagents in mice of many different genetic backgrounds and never observed differential deletion. In addition, it would be rather amazing if *ΔX*, *ΔY*, and *Sma* transgenic mice, with their very different expression patterns, exhibited exactly the same defective elimination of a rare subpopulation.

Our data that none of the mutant transgenes prevent insulitis may suggest another mechanism. Protection could require display of I-E on a certain type of cell and the *Sma*, *ΔX*, and *ΔY* transgenic mice might all lack I-E expression on that particular cell [even though no such defect has been recognized in extensive analyses (*14*)]. Alternatively, protection could require display of the E molecule on two or more types of cells. Extensive complementation and adoptive transfer experiments need to be performed to distinguish between these possibilities. In the meantime, we note that the one functional defect common to the *ΔX*, *ΔY*, and *Sma* transgenic mice is an inability to prime I-E–restricted T cells in lymph node proliferation assays (*14*). The problem is different in each case: *ΔX* mice are not able to positively select I-E–restricted T cells; *ΔY* mice lack I-E molecules on their macrophages, which appear to be required for initiating a lymph node T cell response; and *Sma* transgenic mice lack I-E on B cells, which are the major presenting cells in the proliferative response (*14, 15*). So we are tempted to suggest that the protection mechanism involves a T cell proliferation response, not unlike the reaction to a foreign antigen. The I-E molecule would then have a positive influence rather than the negative one usually proposed.

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