isolated microdissection clones from bands 5q34-q35 and used them to identify 39 cosmid clones [D. Saltman *et al.*, *Nucleic Acids Res.* **20**, 1401 (1992)], which then were oriented by FISH analysis of metaphase chromosomes from the SUP-M2 and SU-DHL-1 t(2;5)-positive cell lines [R. Morgan *et al.*, *Blood* **73**, 2155 (1989)]. Seventeen clones mapped centromeric and 22 clones telomeric to the breakpoint; clones from these groups were oriented relative to one another by two-color metaphase FISH analysis. The estimated genomic distance between the two cosmids that flanked the breakpoint most closely was 290 kb by interphase FISH analysis [J. B. Lawrence, R. H. Singer, J. A. McNeil, *Science* **249**, 928 (1990); B. Trask *et al.*, *Am. J. Hum. Genet.* **48**, 1 (1991)]. Probes prepared from these cosmids did not detect rearranged restriction fragments by Southern (DNA) blot analysis of pulsed-field gels containing DNA from t(2;5)-positive cell lines.

6. The genomic fragment p16-3/1.2S is located immediately centromeric to the chromosome 5 breakpoint, whereas fragment p21-3/3E lies just telomeric to the break. Both probes identified a 1.6-kb transcript in Northern analysis of RNAs prepared from t(2;5)-positive and t(2;5)-negative cell lines; in addition, p16-3/1.2S hybridized to a 2.4-kb transcript found only in t(2;5)-positive cells.
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19. The faint ~4-kb RNAs detected by pS1.2 in the t(2;5)-positive cells represent cross-hybridization of the probe with the 28*S* ribosomal RNA.
20. The nucleotide sequence of *NPM-ALK* (accession number UO4946) has been deposited in GenBank. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Diversity of Endogenous Epitopes Bound to MHC Class II Molecules Limited by Invariant Chain

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The invariant chain (Ii) binds nascent major histocompatibility complex (MHC) class II molecules, blocking peptide binding until the complex dissociates in the endosomes. This may serve to differentiate the MHC class I and II antigen presentation pathways and enable class II molecules to efficiently bind peptides in the endosomes. This hypothesis was addressed by probing spleen cells from a combination of knock-out and transgenic mice with a large panel of T cell hybridomas. The Ii molecule blocked the presentation of a range of endogenously synthesized epitopes, but some epitopes actually required Ii. Thus, the influence of Ii on presentation does not follow simple rules. In addition, mice expressing Ii were not tolerant to epitopes unmasked in its absence, a finding with possible implications for autoimmunity.

The primary function of MHC class II molecules is to present peptides to $CD4^+$ T cells, often those derived from foreign, endocytosed proteins and encountered by class II molecules as they traffic through endocytic vesicles (*1*). In theory, peptides from exogenous antigen must compete for presentation with the vast quantities of endogenous proteins that transit through the endoplasmic reticulum (ER) and Golgi compartments and are known to engender MHC-binding peptides (*1–3*). It has been hypothesized that efficient loading of class II molecules in the ER and Golgi is prevented by Ii, a transmembrane protein that targets class II molecules to endosomes (*4–6*) and interferes with MHC class II–peptide interactions (*7*). However, this hypothesis has been challenged as unnecessary because many class II–peptide interactions are inefficient at the neutral pH of the ER and Golgi, requiring the more acidic conditions of the endosomes (*8*). In addition, recent studies on mice lacking Ii showed that, in the absence of Ii, class II molecules have a conformation which suggests that they are empty of peptide (*5, 6*). We have evaluated the effect of Ii on the presentation of endogenous proteins, using bona fide antigen-presenting cells (APCs) from mice derived by crossing a line devoid of Ii with transgenic lines expressing a segment of myelin basic protein (MBP) under the control of an MHC class II gene promoter. Processing and presentation of the various epitopes within the transgenic MBP segment was read out with a panel of MBP-reactive T cell hybridomas.

Mutant mice lacking the invariant chain (Ii^o) have been described previously (*5*). When crossed onto the $H\text{-}2^s$ genetic background, the Ii^o mutation leads to somewhat reduced A^s surface levels but full ability to present exogenous peptide, as when carried on the $H\text{-}2^b$ background (*5, 6*). Transgenic mice expressing a fragment of MBP (Tg) were produced as part of another study and will be described in detail elsewhere (*9*). As illustrated in Fig. 1A, they produce a chimeric protein consisting of residues 84 to 105 of MBP [MBP(84–105)] inserted between position 43 and 44 of hen egg lysozyme (HEL), selected as a relatively innocuous carrier. This MBP fragment contains several overlapping epitopes recognizable in the context of A^s and characterized extensively in past studies (*10*) and that can be distinguished with the peptides shown in Fig. 1B. The chimeric proteins are expressed in secreted form with the HEL signal sequence (sLM43) or are membrane-anchored as a result of fusion with the transmembrane and cytoplasmic domains of influenza A virus hemagglutanin (mLM43). Expression is driven by the MHC class II Eα promoter, which directs transcription in the thymus and in

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peripheral APCs like B cells, macrophages, and dendritic cells (*9, 11*). Transgenic mice on the *H-2*^{s} genetic background were appropriately mated with Ii-deficient mice to produce Ii$^+$ and Ii° littermates that express the transgenes and are *H-2*^{s} homozygous. Spleen cells from these animals were tested for the ability to present endogenous HEL-MBP protein to a panel of A^s-restricted T cell hybridomas specific for epitopes within MBP(84–104) (*12*). These hybridomas were raised by injection of MBP(84–104) and restimulation of draining lymph node cells in vitro with bovine MBP before fusion. From these fusions we selected the hybridomas that responded to MBP(84–104) presented by mouse fibroblast (L cell) transfectants expressing A^s. Their fine specificity was determined with the peptides shown in Fig. 1B.

Presentation of exogenously supplied antigen—either natural MBP, bacterially synthesized HEL-MBP fusion protein, or MBP(84–104)—was evaluated in parallel with endogenously synthesized antigen (*13*). Many of the T hybridomas (30 of 57) reacted to exogenous peptide but not to either form of the transgene-encoded protein, whether or not Ii was present. Splenic APCs must lack the ability to process endogenously synthesized protein to generate the particular epitopes recognized by these T cells. Results for a selection of hybridomas that did recognize endogenously synthesized MBP fragment are summarized in Fig. 2; representative dose-response curves are presented in Fig. 2A, and data from a wider set are tabulated in Fig. 2B. Several conclusions can be drawn from the data.

1) T cells that recognize the endogenously synthesized MBP fragment may do so only in the absence of Ii (4C1), in its presence or absence (3A1), or preferably in its presence (3A5). Considering our entire panel of T hybridomas, strong or absolute preference for the absence of Ii is more frequent (16 of 27) than indifference to (5 of 27) or preference for (6 of 27) Ii.

2) A wider range of epitopes can be presented in the absence than in the presence of Ii. While those T hybridomas that respond to Ii$^+$ APCs are limited to one of the MBP specificity groups described in Fig. 1B (group 1, 11 of 11 hybridomas), those that prefer or require Ii° APCs belong to all three of the specificity groups (group 1, 5 of 16 hybridomas; group 2, 3 of 16; group 3, 8 of 16). In addition, one hybridoma recognizes yet another epitope with Ii° APCs, present in the large peptide (84 to 104) but not in any of the subfragments tested.

3) There is a correlation between epitope generation and the form of the chimeric protein: those epitopes that are recognized only in the absence of Ii are not generated from the membrane-anchored protein by the splenic APCs from transgenic mice [although transfected L cells expressing higher levels are quite efficient at processing the membrane-anchored protein to generate these epitopes (9)].

4) Almost all T cells behave similarly when presented with exogenously supplied MBP, irrespective of the form. Most important, there is no correlation between the

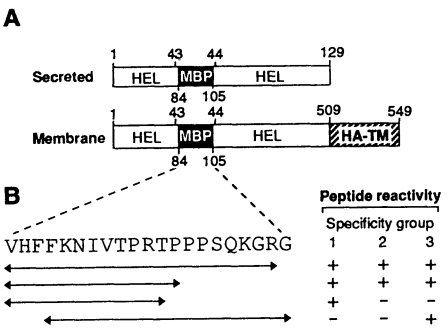

Fig. 1. Expression of a chimeric endogenous protein. (**A**) Schematic representation of the transgenic fusion proteins. Sequences coding for MBP(84–104) were inserted between sequences encoding residues 43 and 44 of an HEL cDNA. A membrane-bound variant of the protein was generated by fusion with sequences specifying the transmembrane and cytoplasmic domains of influenza A/PR8 virus hemagglutinin. The constructs were expressed under the control of the MHC class II Eα promoter (*11*) in the transgenic lines E43.30 (expressing sLM43) and EH43.69 (mLM43). These lines, on the *H-2*^{s} background, were crossed with the Ii° line (*5*) for two generations to obtain *H-2*$^{s/s}$, transgene-positive animals that were heterozygous or homozygous for the Ii° mutation. (**B**) MBP peptide sequence and definition of specificity groups of A^s-restricted, MBP-specific T cell hybridomas. The sequence of the MBP insert 84 to 104 [according to the numbering of the mouse sequence of MBP (*22*)] is shown above double-ended arrows that represent the peptides (residues 84 to 104, 84 to 96, 84 to 95, and 87 to 105) used to establish the specificities of T hybridomas. These peptides were used at a saturating dose of 5 μg/ml (approximately 500-fold above the threshold of detection). The distinctions between the presence and absence of stimulation were sharp, in keeping with previous work showing the strict distinction between epitopes in this region imparted by Pro^{96} (*10*). They fell into one of three specificity groups on the basis of recognition of these peptides (*23*).

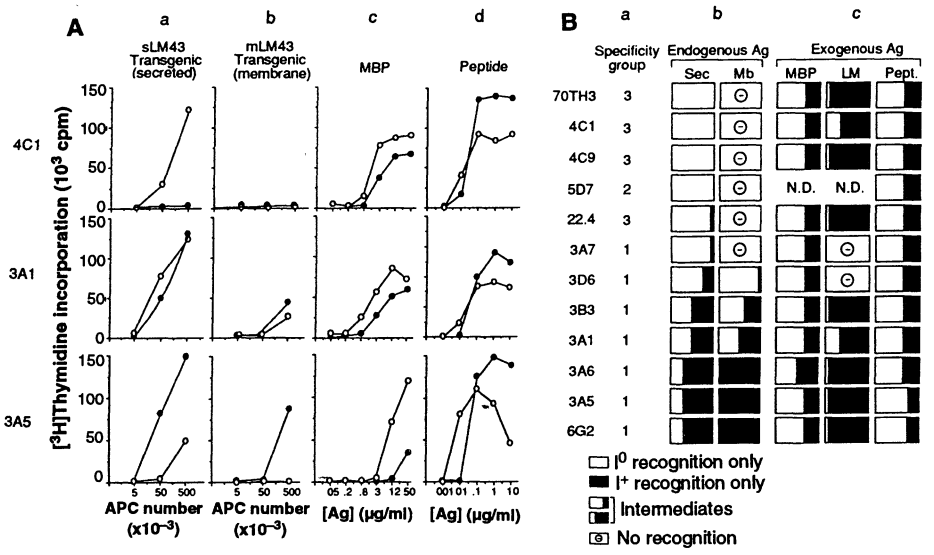

Fig. 2. MBP-specific T cell hybridomas have different Ii requirements for recognition of endogenous MBP fragment. (**A**) Representative dose-response curves for selected T hybridomas 4C1, 3A1, and 3A5. Open circles, Ii° spleen; closed circles, Ii$^+$ spleen. Results are shown as CTLL-2 proliferation readouts of IL-2 production. (a and b) Titrations of spleen cells expressing either secreted or membrane-bound forms of the LM43 transgene (without the addition of exogenous antigen). (c) Titration of the 21.5-kD isoform of bovine MBP (Sigma; repurified by reversed-phase high-performance liquid chromatography on a Vydac C4 column). (d) Titration of the synthetic peptide MBP(84–104). These results are representative of at least two independent experiments for each of the T hybridomas shown. (**B**) Recognition of the endogenously synthesized or exogenous forms of protein, for a set of 12 T hybridomas. (a) Epitope specificity, determined as described in the legend to Fig. 1. (b) Stimulation by the endogenous (secreted or membrane) forms of the chimeric protein in transgenic spleen cells (sLM43 and mLM43, respectively). (c) Activation by exogenously supplied antigens [21.5-kD bovine MBP, *E. coli*–produced chimeric protein (LM), and MBP(84–104) peptide]. Symbols indicate the preference of individual hybridomas for presentation by Ii$^+$ as compared with Ii° spleen cells: white rectangles represent an absolute preference for Ii° cells; black rectangles, an absolute preference for Ii$^+$ cells; double-shaded rectangles, recognition of both Ii$^+$ and Ii° cells, with the relative proportions of white or black representing relative efficiencies of presentation by Ii° or Ii$^+$ cells; ○, no recognition in either APC context.

capacity of a given T cell to respond to the exogenous as compared with the endogenously synthesized fusion proteins. This is strong evidence that the transgene-encoded MBP fragment is indeed presented from an endogenous pool rather than by reuptake after secretion or shedding, a point also supported by our inability to detect the chimeric protein in serum or culture supernatants by antibody-based techniques or coculture experiments (*9*). It also implies that distinct processing pathways are used by the exogenous and endogenous forms, as shown in other systems (*3*, *14*).

Given the ability of Ii° APCs to present supplementary epitopes, it was of interest to see whether they could reveal self-reactive T cells in Ii^+ mice that express the MBP fragment in the thymus and should therefore be tolerant to it. The panel of T hybridomas that were available to us came from non-Tg or mLM43 Tg animals, both expressing Ii. Perhaps surprisingly, it was possible to isolate MBP peptide– and natural protein–reactive hybridomas from both types of mice with similar efficiencies (*9*). Hybridomas from non-Tg, but not from Tg, animals responded to transgene-encoded, endogenously synthesized MBP offered by Ii^+ APCs (Table 1), indicating that the latter are effectively tolerant to true self-epitopes, although they can respond effectively to MBP peptide or natural MBP protein (*9*). In contrast, hybridomas from both non-Tg and Tg animals could react to the transgene-encoded MBP fragment presented by Ii° APCs. For the transgenics, these included three hybridomas from each of two mice, representing all three of the specificity groups defined in Fig. 1B. Thus, additional self-epitopes can be revealed in the absence of Ii.

We have used a large panel of T hybridomas to evaluate the influence of Ii on the presentation of endogenous proteins. The little information previously available was inconsistent: one T hybridoma recognized endogenously synthesized HEL better in the presence of Ii (*15*), and one hybridoma responded equally well to presentation of L^d in the presence or absence of Ii (*16*). It was not clear whether the inconsistency reflected differences in T hybridomas, antigen, or some feature of the Ii-transfected APC system. Here, we find that Ii does serve, in bona fide APCs, to prevent or reduce the presentation of epitopes derived from an endogenous protein. The more efficient presentation of certain endogenous protein epitopes by Ii° APCs is particularly notable considering that there are many fewer class II molecules on such cells and that those that are present do not have a conformation indicative of tight peptide binding (*5*, *6*).

Yet, Ii is not inhibitory in all instances, the presentation of one MBP epitope strongly preferring Ii in most cases. How can one account for this dichotomy? It could be that the generation of different protein fragments takes place in distinct cellular compartments containing distinct proteolytic enzymes. Indeed, Vidard *et al.* (*17*) have shown that diverse protease inhibitors increase or decrease presentation of ovalbumin epitopes to a panel of T cells, the effect depending on the particular T cell and epitope. Ii would thus prevent the binding of most peptides generated in the ER, but its targeting function would be necessary for presentation of other peptides generated in the endosomes. In summary, the influence of Ii on presentation of endogenous antigens follows no simple rule.

Whatever the explanation for the dichotomy, it is interesting to consider whether the diversification of epitopes presented in the absence of Ii has any implications for autoimmunity. As demonstrated here, APCs lacking Ii can reveal the presence of self-reactive T cells in an animal. Such cells would not have been eliminated during tolerance induction because they react to an epitope not presented by Ii^+ APCs, clearly the majority in the thymus and periphery. Ii and class II molecules are normally synthesized coordinately. There are a few reported examples of a dissociation of their expression levels, although it is not clear in most cases whether this is at the level of biosynthesis (*18*). Probably the most convincing evidence of dissociated expression is Vidali *et al.*'s recent demonstration that murine gut epithelial cells express class II molecules but are deficient in Ii (*19*). Unmasking of self-reactive T cells by any Ii-negative, class II–positive cells could play a role in some forms of autoimmune disease, particularly when "non-professional" APCs might be involved. Such a scenario would provide a mechanism for the revelation of "cryptic determinants" as discussed by Sercarz and colleagues (*20*).

Table 1. Recognition of Ii^+ or Ii° Tg spleen APCs by T cell hybridomas derived from Tg or non-Tg Ii^+ mice. T cell hybridomas specific for MBP(84–104) were derived from either mLM43 Tg or non-Tg mice, both Ii^+. The table groups the individual hybridoma according to whether it recognizes an MBP epitope presented by sLM43 spleen cells in the presence of Ii (Ii^+ Tg column) or in its absence (Ii° Tg column) or whether it is unable to recognize the transgene-encoded protein at all (column labeled neither). No exogenous antigen was added.

Hybridoma source	Number of T hybridomas recognizing splenic APCs from		
	Ii^+ Tg	Ii° Tg	Neither
Nontransgenic	11	10	11
Transgenic	0	6	19

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