

The T Cell Response of HLA-DR Transgenic Mice to Human Myelin Basic Protein and other Antigens in the Presence and Absence of Human CD4

By Daniel M. Altmann, Daniel C. Douek, Arlene J. Frater, Colin M. Hetherington, Hidetoshi Inoko, and James I. Elliott

From the Transplantation Biology, MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, United Kingdom

Summary

Analysis of HLA class II transgenic mice has progressed in recent years from analysis of single chain HLA class II transgenes with expression of mixed mouse/human heterodimers to double transgenic mice expressing normal human heterodimers. Previous studies have used either HLA transgenic mice in which there is a species-matched interaction with CD4 or mice which lack this interaction. Since both systems are reported to generate HLA-restricted responses, the matter of the requirement for species-matched CD4 remains unclear. We have generated triple transgenic mice expressing three human transgenes, *DRA*, *DRB*, and *CD4*, and compared HLA-restricted responses to peptide between human-CD4⁺ (Hu-CD4⁺) and Hu-CD4⁻ littermates. We saw no difference between Hu-CD4⁺ and Hu-CD4⁻ groups, supporting the notion that for some responses at least the requirement for species-matched CD4 may not be absolute. Evidence for positive selection of mouse T cell receptors in HLA-DR transgenic mice came both from the acquisition of new, HLA-restricted responses to various peptides and from an increased frequency of T cells using the TCR V β 4 gene segment. An important goal with respect to the analysis of function in HLA transgenic mice is the clarification of mechanisms which underpin the recognition of self-antigens in human autoimmune disease. As a first step towards 'humanized' disease models in HLA transgenic mice, we analyzed the responses of HLA-DR transgenic mice to the human MPB 139-154 peptide which has been implicated as an epitope recognized by T cells of multiple sclerosis patients. We obtained T cell responses to this epitope in transgenic mice but not in nontransgenic controls. This study suggests that HLA transgenic mice will be valuable in the analysis of HLA-restricted T cell epitopes implicated in human disease and possibly in the design of new disease models.

The mapping of patterns of MHC restriction, epitope recognition and T cell effector function in the murine immune response has generally been determined by experimental *in vivo* priming. Analysis of human T cell responses has necessarily involved a different approach: lines and clones derived from natural priming have been analyzed *in vitro*, assessing HLA-restriction for example using various means including antibody blocking and B-lymphoblastoid cell lines (BLCL)¹ or HLA transfectants as antigen presenting cells. These approaches, though valuable, have left functional questions such as the *in vivo* effector activity of human clones of particular specificities or the functional basis for HLA associations with autoimmune disease difficult to confront. The generation of HLA transgenic mice (as well as complemen-

tary human TCR transgenic mice) has therefore been an important step towards the creation of *in vivo* models for the functions of human molecules.

HLA class I transgenic mice have been available for some years and have allowed the analysis of HLA-restricted responses with variable success. HLA-B27/ β 2m double transgenic mice were shown to make HLA-restricted responses to influenza virus and Sendai virus (1, 2). When expressed in transgenic rats at high copy number, HLA-B27 was associated with a disease phenotype reminiscent of the B27-associated spondyloarthropathies, although the role of HLA-restricted T cells in pathogenesis is unknown (3, 4). However, mice transgenic for a number of HLA class I transgenes did not have demonstrable HLA-restricted T cells at significant frequencies and did not recognize HLA-disparate cells at a higher frequency than the normal, low level of murine xenoreactivity to human targets (5, 6). This could have been attributable either to some structural constraint on positive selection of mouse T cell

¹ Abbreviations used in this paper: B-LCL, B-lymphoblastoid cell lines; DP, double positive; MBP, myelin basic protein; MS, multiple sclerosis.

receptors by human class I molecules or to the requirement for a species-matched interaction between CD8 and class I in order for T cell activation to occur. The latter proposal is supported by the finding that there is strong recognition by mouse T cells of transgenic cells carrying chimaeric molecules where a species-matched class I/CD8 interaction is restored by splicing the murine $\alpha 3$ domain into a human class I molecule (7).

With respect to HLA class II transgenic mice, a number of laboratories including ours showed that single human class II α or β chains expressed from transgenes could pair with murine partner chains to give a functional molecule capable of inducing positive or negative selection of mouse T cell receptors (8–12). One laboratory has produced data showing that HLA-DR and DQ transgenic lines can make HLA-restricted responses to various antigens in the absence of a Hu-CD4 molecule (13, 14). Nevertheless, the extent to which murine CD4 can interact with HLA class II molecules has remained a contentious issue (15–17).

We here report HLA-DR1 transgenic mice made with relatively large fragments of genomic sequence to retain faithful tissue expression of the molecule in mice which either have or lack a human CD4 transgene. The mice have been used to investigate responsiveness to various antigenic peptides including, as a step towards the analysis of HLA class II transgenic disease models, a human myelin basic protein (MBP) epitope commonly recognized by multiple sclerosis (MS) patients (18, 19).

Materials and Methods

Transgenic Lines. HLA-DR1 transgenic mice were generated by coinjection of inserts from two cosmid clones, MANN 4.2 which carries the full *HLA-DRA1*0101* gene and several kilobases of flanking sequence (20) and pWE15DR1B1 which carries the full *HLA-DRB1*0101* gene and several kilobases of flanking sequence. Expression of the former construct has previously been described by us (11, 12) and the potential for expression of the DRB cosmid was confirmed by cotransfection with DRA into mouse L cells (H. Inoko, unpublished observations). Transgenic mice were generated by coinjection of the DRA gene as a 24-kb BamHI fragment and of a 22-kb NotI fragment carrying the DRB gene into fertilized FVB/N oocytes (21). The work reported here is based on progeny from one of the founders which was found to have integrated multiple copies of the two transgenes. HLA transgenic mice were initially characterized by Southern hybridization of BamHI-digested genomic DNA to a DRB cDNA probe. To do this, 20 μ g of tail DNA was digested to completion with BamHI, run on an agarose gel and blotted onto Hybond-N (Amersham International, Amersham, UK), before hybridizing to a 510-bp DRB 5' cDNA probe at 65°C, then washing at high stringency. Blots were exposed to XAR-5 film overnight. Hu-CD4 transgenic mice have previously been described (22). Briefly, they were generated with a construct whereby a full-length cDNA for the human CD4 molecule is under the control of a mouse class I H-2K promoter. Once lines had been established the identity of transgenic animals among progeny was assessed by PCR. For HLA-DR we used a pair of primers corresponding to amino acid residues 18–24 and 84–93 of HLA-DRB1*0101 (sense primer: TTC TTC AAC GGG ACG GAG CGG GTG; antisense primer: CTG CAC TGT GAA

GCT CTC ACC AAC). Cosegregation of the DRA transgene was initially checked by hybridization of a DR α cDNA probe to Southern filters of genomic DNA and thereafter by PCR with the following DRA primers. Sense: the primer pair used for detection of human CD4 was sense primer: AGG CCT CCA GCA TAGT; anti-sense primer: CCT GAT GCA ACT TTC CTG. Mice were maintained as hemizygotes to allow functional comparison of transgene positive and negative mice from individual litters.

Flow Cytometry. Splenocytes were resuspended in phosphate-buffered saline containing 0.5% BSA and incubated on ice in the presence of a saturating concentration (25 μ g/ml) of the following mAbs which were used as affinity purified and biotinylated IgG: L243 (anti-DR α) (23), TDR31.1 (anti-DR β) (24), TAL 14.1 (anti-DR β) (25, 26), M5/114.15.2 (anti-H-2A), Leu 3a (anti-human CD4), and KT174 (anti-mouse CD4). Phycoerythrin (PE)-streptavidin (Becton Dickinson, Cowley, UK) was used as the second layer. 8,000 events were analyzed for each sample on a FACScan (Becton Dickinson). For three-color analysis of CD4⁺8⁺ double positive (DP) thymocytes, cells were stained with anti-mouse CD4-PE (CALTAG Labs., South San Francisco, CA), KT15 anti-mouse CD8-FITC and biotinylated Leu 3a, with streptavidin-tricolor as second layer. Cells were tightly gated on mouse CD4⁺CD8⁺ DP's (FL1 and FL2) and these cells were then analyzed for FL3 fluorescence. For the evaluation of the percentage of T cells bearing specific V β chains, 10⁶ mesenteric lymph node cells from 3-mo-old mice were incubated for 45 min at 4°C with FITC-conjugated anti-CD4 (KT6) and anti-CD8 (KT15), and biotinylated anti-V β antibodies (V β 3, KJ25 [27]; V β 4, KT4 [28]), V β 8.1,2,3, F23.1 [29]; V β 5.2, MR9.8 [30]; V β 13, MR12-4 [31]; and V β 10, B20.5 [12]), washed three times and incubated for 20 min at 4°C with PE-streptavidin (Becton Dickinson). Cells were analyzed on a FACScan using LYSIS II software and gated for live cells on the basis of forward and side scatter. The CD4⁺ and CD8⁺ populations were separated on the FL1 axis by virtue of their relative brightness and could then be gated for statistical analysis of V β staining in each compartment. Statistical significance was calculated by Student's *t* test.

Immunocytochemistry. Frozen sections of transgenic and non-transgenic mouse thymus were fixed with acetone and stained with biotinylated L243 (anti-DR α) or TAL 14.1 (anti-DR β). They were probed with streptavidin-horseradish peroxidase (Dako Corp., Carpinteria, CA), developed with diaminobenzidine (Dako) and counterstained with Mayer's hematoxylin (Sigma Chemical Co., St. Louis, MO).

T Cell Proliferation Assays. Young adult, age, and sex-matched mice were primed in each hind footpad with 50 μ g of each of the indicated antigenic peptides in complete Freund's adjuvant. 8 d later (except where indicated otherwise) popliteal lymph node cells were removed and assayed for T cell proliferation at 4 \times 10⁵ cells/flat-bottom well. Plates were cultured for 72 h in HL1 serum-free medium (New Brunswick Scientific Ltd, Hatfield, UK) containing penicillin, streptomycin, glutamine, and 2-mercaptoethanol. Wells were pulsed for the final 6 h with 1 μ ci [³H]thymidine before being harvested onto filtermats for liquid scintillation counting in an LKB Betaplate counter. Unless stated otherwise, results are expressed as the Δ cpm of the mean from cultures with antigen minus the mean from cultures without antigen, where background counts in the absence of antigen were always less than 1,000 cpm.

Generation of T Cell Lines. Popliteal lymph node cells from HA 307-319 immunized HLA-DR1 transgenic mice were removed at day 11 after immunization and cultured at 2 \times 10⁶ cells/ml in 12-well plate (Costar Corp., Cambridge, MA). After a further 10 d they were restimulated using irradiated HLA-DR1⁺ mouse splenocytes and peptide at 50 μ g/ml. 3 d after restimulation the

medium was supplemented with 20 $\mu\text{g}/\text{ml}$ recombinant IL-2. This cycle of restimulation was repeated a further three times. Lines were tested against a panel of APC 8–10 d after the previous restimulation.

Antigen Presenting Cell Lines for Analyzing HLA Restriction of T Cell Lines. Various cell lines were tested for antigen presentation to T cell lines, using 4×10^5 APC plus 1×10^4 line T cells per well. The human B cell lines PGF (DRw15 homozygous B-LCL), HOM-2 (DR1Dw1 homozygous B-LCL), and the class II null mutant, RJ2.2.5 were all maintained in RPMI 1640 supplemented with 10% FCS penicillin, streptomycin, and glutamine. Mock-transfected (vector only) mouse L cells and their DR1-transfected counterpart (26) were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS penicillin, streptomycin, glutamine, and 500 $\mu\text{g}/\text{ml}$ Hygromycin B (Calbiochem-Novabiochem Corp., La Jolla, CA). All APC were treated with 40 $\mu\text{g}/\text{ml}$ mitomycin c (Sigma) at 37° for 40 min before washing and adding to microtitre plates. T cell line/APC assays were conducted in HL1 medium for 72 h as described above.

Results

Characterization of HLA-DR1 Transgenic Mice. Mice were initially characterized by Southern hybridization of BamHI-digested genomic DNA to *DRA* and *DRB* cDNA probes. Fig. 1 *a* shows an example of this analysis with a transgenic mouse shown in track A and human genomic DNA in track

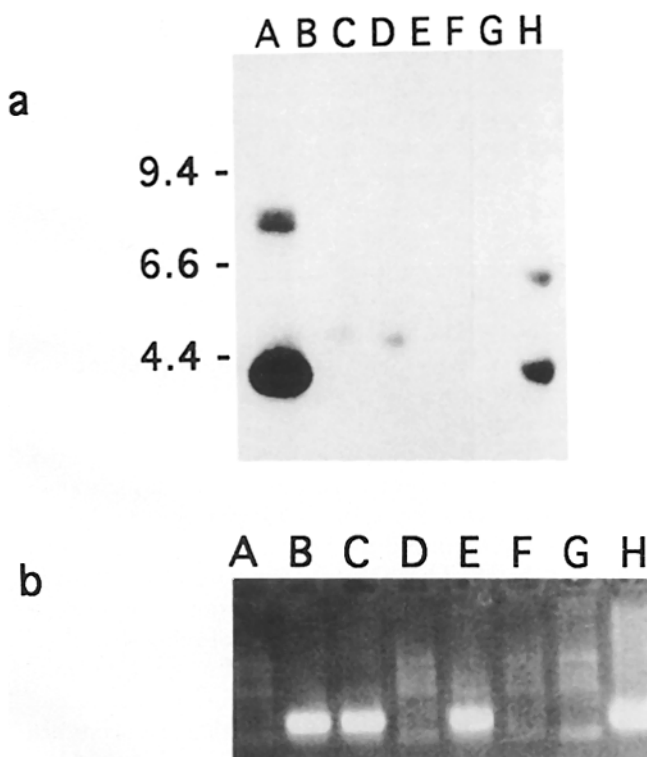


Figure 1. Genotyping of transgenic mice. (a) Southern analysis of genomic DNA from an HLA-DR⁺ litter hybridized with a DR β cDNA probe. Lanes A–F are offspring from the litter where A shows a positive mouse. Lane G, non-transgenic mouse negative control; lane H, human genomic DNA positive control. (b) PCR analysis of the same litter using PCR primers for human CD4. The tracks are as in a.

II. The strong band to which the DR β cDNA hybridizes is the ~ 4 -kb BamHI genomic fragment carrying exons 2 and 3 of *DRB*. All mice which were positive for this band by Southern blotting were also found to be positive by PCR for both *DRB* and *DRA*. The human CD4 transgene was crossed onto HLA-DR1 transgenic from an independent founder and so segregated independently of the DR transgenes in crosses between the two transgenic lines. PCR analysis of the CD4 transgene is shown in Fig. 1 *b*.

When tissues from HLA-DR transgenic mice were analyzed for HLA-DR mRNA by Northern analysis, we obtained the result previously reported (11); HLA-DR mRNA was expressed in spleen, lymph node and thymus with a very small amount of transcript in kidney and lung while brain, heart, and testis were negative (data not shown). We then analyzed surface expression of the human heterodimer on FACS (Fig. 2). Splenocytes of triple transgenic mice or non-transgenic

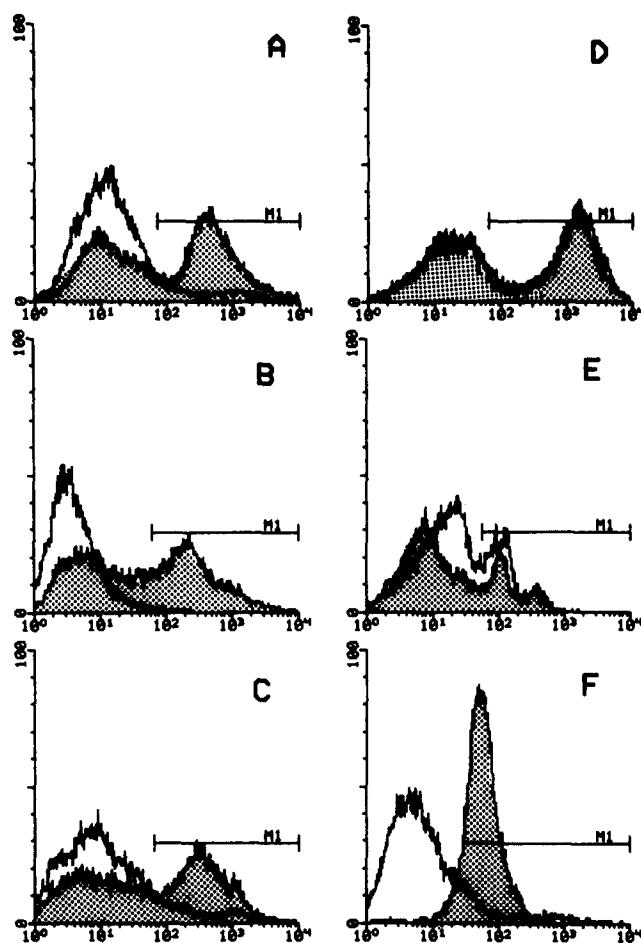


Figure 2. FACS analysis of splenocytes from HLA-DR/Hu-CD4 transgenic mice. In each panel, staining of cells from a triple-transgenic is shown by the stippled histogram and of cells from a negative littermate by the unshaded histogram. The x axis shows log FL-2 brightness and the y axis cell number. The bar marked M1 in each case shows the population of cells with specific fluorescence brighter than obtained when an isotype-matched irrelevant first layer was used. The antibodies are as follows: A, L243 (DR α); B, TDR 31.1 (DR β); C, TAL 14.1 (DR β); D, M5/114.15.2 (H-2A); E, KT174 (Mu-CD4); F, Leu 3a (Hu-CD4).



littermates were stained with mAbs against HLA-DR and CD4. One mAb against DR α (L243, panel A) and two different mAbs against non-polymorphic epitopes on DR β (TDR31.1 and TAL, 14.1, panels B and C) each showed clear staining of a distinct, brightly stained sub-population of cells. Expression was comparable with the brightness of staining obtained using the anti-H-2A mAb M5/114.15.2 (panel D). In some experiments lymph node cells or peripheral blood were stained and also found to be strongly positive for the transgenes (data not shown). No cross-reactive staining of mouse class II was seen in cells from non-transgenic littermates (*unshaded histograms*). The expression level of hu-CD4 (F) and mouse CD4 (E) was comparable, although Hu-CD4 was expressed by all cells, as previously described (22).

We examined transgene expression in various tissues by immunocytochemistry. Fig. 3 shows the expression pattern in transgenic thymus which was stained with no first layer mAb (*top*), L243 (*middle*), or TAL 14.1 (*bottom*). Staining was found in a classical class II pattern with both antibodies, namely a reticular distribution in the cortex, reflecting expression on cortical epithelium and a more confluent pattern in the medulla, reflecting expression on dendritic cells and a proportion of epithelial cells.

T Cell Responses of Transgenic Mice to Immunization with Peptide. We examined T cell responses to various peptidic antigen fragments which have been reported to be recognized by T cells in HLA-DR1 positive individuals. We found that transgenic mice could respond to the 307-319 epitope of influenza haemagglutinin (32) while non-transgenic littermates could not (Fig. 4 a). A recent report in which T cell hybridomas from chimaeric HLA class II/H-2E transgenic mice were analyzed reached similar conclusions (17). Although we have found that some H-2E⁺ strains of mice can respond to HA 307-319 (data not shown), the only class II molecule available to non-transgenic littermates in this study, H-2A^a, does not elicit a T cell response to HA 307-319.

A number of studies have sought to identify epitopes of MBP recognized by T cell clones from MS patients. While some studies focused on clones restricted via products of the disease associated HLA-DRw15 DQw6 haplotype, many other HLA types have also been examined since the association is not exclusive. Two groups analyzing responses in HLA-DR1 positive individuals identified an epitope in the region of MBP 139-154 as being of possible relevance to disease, although this epitope is also recognized in association with other alleles (18, 19). In view of the desirability of being able to test disease-implicated myelin epitopes in HLA transgenic mice in which pathogenesis could be followed (for example after using HLA-restricted peptides in the induction of experimental allergic encephalomyelitis, we examined the response to MBP

Figure 3. Immunohistochemical analysis of transgenic mouse thymus cryosections. Monoclonal antibodies were biotinylated and probed with streptavidin-horseradish peroxidase. (*Top*) No first layer antibody (brown specks are non-specific uptake of streptavidin); (*middle*) L243; (*bottom*) TAL 14.1.

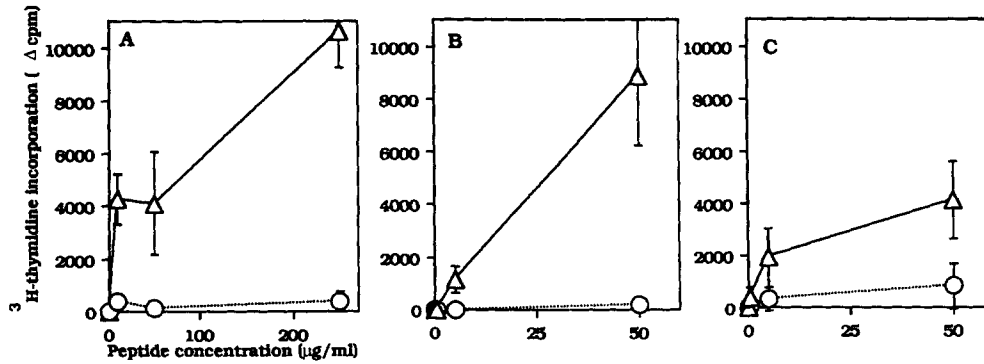


Figure 4. T cell proliferation to antigenic peptides in HLA-DR transgenic mice. Responses of popliteal lymph node cells were analysed in mice which had been immunized with (A) HA 307-319, (B) MBP 139-154, or (C) tetanus toxoid 830-843. Responses in HLA-DR transgenic mice are shown as open triangles and in non-transgenic littermates as open circles. Results are shown as the mean Δ cpm \pm SE from three mice per group assayed individually. Background counts of T cells and medium were $<1,000$ cpm.

139-154 in HLA-DR1 transgenic mice (Fig. 4 b). Transgenic mice responded to the peptide while non-transgenic littermates did not. We saw no evidence of any neurological disease in MBP 139-154 immunized mice (data not shown), therefore disease studies will require the transgene to be expressed on a different genetic background as FVB/N mice are resistant to induction of EAE.

Other peptides which we tested in HLA-DR1 transgenic mice either elicited weaker responses or provoked no response in either group. Fig. 4 c shows the response to tetanus toxoid 830-843, an epitope recognized by human T cells from individuals of several different HLA-DR types (33). When mice were immunized with this peptide both transgenics and negative littermates made relatively low responses, although responses were significantly greater in the transgenic group E. pertussis toxin 27-39 (34), a further epitope which can be presented via HLA-DR1 to human T cells, elicited no responses from transgenics or negative littermates (data not shown).

HLA Restriction of Responses To Peptide. Although new responses which can be found in HLA transgenic mice but not in negative littermates imply HLA restriction of mouse T cells, we confirmed this formally using a panel of human or HLA transfected antigen presenting cells (APC). T cell lines were generated from immunized mice by four rounds of in vitro stimulation with irradiated transgenic splenocytes and peptide. These lines were then tested against a panel of

APC. Fig. 5 shows the response of a T cell line specific for HA 307-319: of the 3 human B-LCL that were tested, the DR1⁺ B-LCL (HOM-2) presents the peptide to the T cell line, but neither an HLA-mismatched B-LCL (the DRw15 homozygous line, PGF) nor an HLA-null mutant (RJ2.2.5) elicit any response. Furthermore, the matched HLA-DR1 transfected L cell could present peptide to the line while its mock-transfected counterpart could not.

Effect of a Human CD4 Transgene on HLA-restricted Responses. In view of conflicting data in the literature over the necessity for a species-matched MHC class II/CD4 interaction for T cell responses, we explored this question by crossing the HLA-DR1 transgenic line and a transgenic line expressing human Hu-CD4 in which a full-length Hu-CD4 cDNA is driven off a mouse class I promoter. Although the CD4 transgene is expressed at physiological levels on all peripheral lymphocytes (reference 22 and see Fig. 2), we were concerned that, since class I is not expressed at high levels on CD4⁺ CD8⁺ DP thymocytes, the transgene may not be expressed at this stage which would be critical for it to influence T cell development. In fact, when thymocytes were stained for mouse CD4 and CD8, then gated on DP cells to examine the Hu-CD4⁺ population within this gate on a third color, it was found that DP thymocytes express the transgene (Fig. 6). When HLA-DR1⁺ Hu-CD4⁺ transgenic mice and HLA-DR1⁺ Hu-CD4⁻ littermates were primed with the HA 307-319 peptide, both groups made equally strong re-

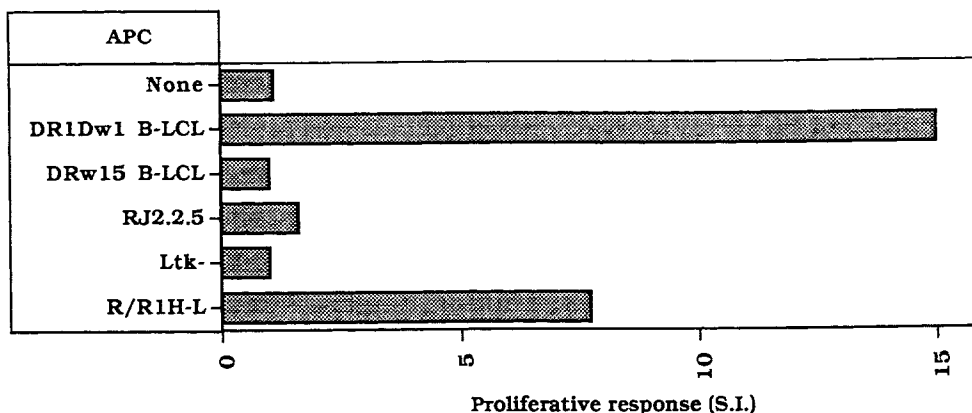


Figure 5. Transgenic mouse T cell lines against HA 307-319 are HLA-DR1 restricted. The T cell line was tested against a panel of APC in a 72-h assay. Proliferation is shown as the stimulation index (S.I.) of T cell + APC + peptide divided by T cell + APC where mean backgrounds without peptide ranged from 193 to 1299 for the various cell combinations.

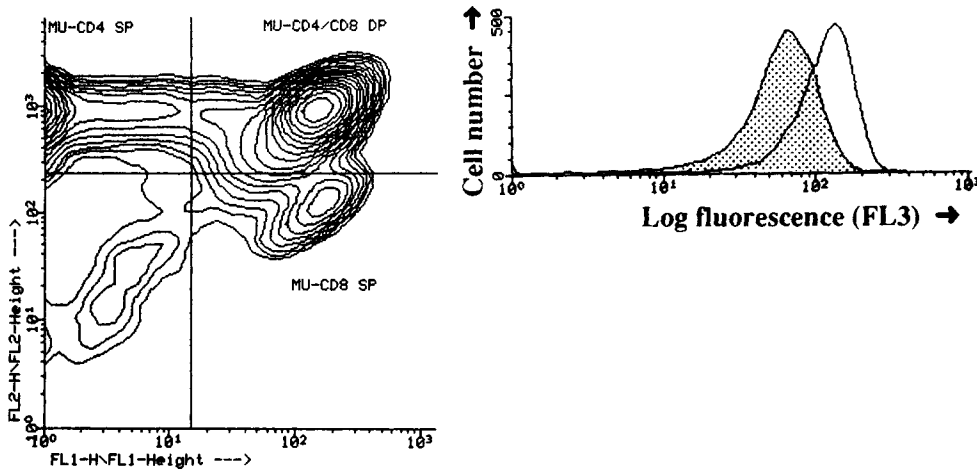


Figure 6. Murine CD4⁺CD8⁺ double positive thymocytes express the Hu-CD4 transgene. Thymocytes from a Hu-CD4⁺ transgenic mouse or negative littermate were tightly gated for DP cells as indicated in the materials and methods and then analyzed on FL-3 for Hu-CD4 expression (Leu 3a). The contour plot on the left shows staining for Mu-CD4 (FL-2) and CD8 (FL-1) used to identify DP cells. The stippled histogram shows staining of cells from the non-transgenic littermate and the open histogram, specific staining in the transgenic mouse using Leu 3a (FL-3).

sponses to the peptide and no additional effect of the Hu-CD4 transgene was evident (Fig. 7).

TCR V β Repertoire Selection in HLA-DR Transgenic Mice. We and others have previously shown that in some cases positive selection on introduction of a new class II molecule can be detected as gross expansions of the T cell population utilizing a particular V β family (12). We therefore analyzed TCR V β usage by mesenteric lymph node cells from transgenic mice and negative littermates (Table 1). A number of V β mAbs tested gave little staining in transgenic or non-transgenic mice indicating either a germline deletion of some V β gene segments in the FVB/N strain or Mtv-mediated deletions (data not shown). Of the other TCR mAbs tested, V β 4⁺ T cells were greatly increased in HLA-DR transgenic mice, the increase being greater in CD4⁺ than in CD8⁺ T cells. Use of V β 13 was also significantly higher in transgenic animals. These are unlikely to be merely compensatory increases following deletion of some V β families via DR1 and Mtv's since a number of other specificities such as V β 5.1 and V β 10 were unchanged. Although we previously shown that V β 10⁺ cells are positively selected in mice carrying H-2E or in HLA-DRA transgenic C57BL/10 mice (12), there was no effect on V β 10 selection in these mice. We then examined superantigen-mediated deletion via the human class II molecule in these mice which carry Mtv's 7, 8, 13 and 17. Pre-

sumably due to the presence of Mtv 13, transgenic mice show a reduction in the percentage of cells bearing V β 3 although this was only partial. This may imply that HLA-DR1 binds Mtv13 poorly.

Discussion

The complexities inherent in analyzing the specificity, function and relevance to disease of human T cell clones have made it an important objective to generate transgenic mice in which various components of human responses such as relevant HLA molecules and TCR's can be expressed. However, it has been unclear until fairly recently whether it would be possible to obtain normal function of the human gene products within the mouse immune system. The major concerns have been the possible requirement for a species-matched HLA class II/CD4 interaction and the possibility that differences between mouse and human TCR variable sequences would preclude the possibility of obtaining responses restricted via human MHC molecules.

Considerable progress has been made in these areas in recent years, first analyzing single chain HLA class II transgenic lines in which the function of mixed mouse/human molecules was studied (8-12), then double transgenic lines in which normal human heterodimers are expressed (13, 14, 17). We have now extended this analysis to look at triple transgenic mice expressing three human transgenes, DRA, DRB, and CD4. The requirement for a species-matched class II/CD4 interaction has proved difficult to dissect, different models producing conflicting results. One of the earliest reports of HLA class II transgene function came from Nishimura and coworkers who showed the acquisition of transgene-dependent responsiveness to streptococcal cell wall antigen, although this was inhibited by antibodies to HLA-DQ, mouse CD4, and H-2A, raising the possibility of re-presentation of antigen via mouse class II (13). It was subsequently demonstrated that cells from these HLA-DQ transgenic mice could trigger allogeneic recognition of the xenoantigen by non-transgenic mouse T cells, the response again being inhibited by antibodies to mouse CD4 (35). These observations

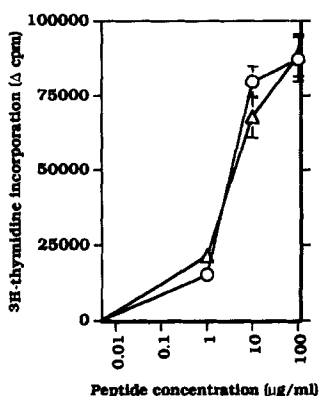


Figure 7. T cell proliferation of HLA-DR transgenic mice to HA 307-319 in the presence or absence of Hu-CD4. Responses of popliteal lymph node cells were analysed in mice which had been immunized 11 d previously with HA 307-319. Responses in Hu-CD4⁺ transgenic mice are shown as open triangles and in Hu-CD4⁻ littermates as open circles. Results are shown as the mean Δ cpm \pm SE from three mice per group assayed individually. Background counts with T cells and medium were <1,000 cpm.

Table 1. TCR V β Usage in HLA-DR Transgenic Mice

	CD4 ⁺ cells (%)		CD8 ⁺ cells (%)	
	Transgene negative	Transgene positive	Transgene negative	Transgene positive
V β 3	4.24 ± 0.44	2.22 ± 0.42	4.16 ± 0.57	2.98 ± 0.60
V β 4	10.50 ± 0.44	16.30 ± 1.39	7.15 ± 0.42	10.46 ± 1.17
V β 10	6.76 ± 1.24	7.10 ± 1.47	1.42 ± 0.54	0.99 ± 0.46
V β 13	18.36 ± 0.80	21.91 ± 1.31	8.25 ± 1.79	10.68 ± 1.34
V β 8.1,2,3	5.69 ± 0.67	6.3 ± 1.42	1.33 ± 0.13	0.90 ± 0.33
V β 5.1	0.50 ± 0.38	1.0 ± 1.0	NT	NT

Data are expressed as mean percentage of five mice \pm SD. Values shown in bold type are significant at $p < 0.001$. NT, not tested.

as well as the more recent one of inhibition of specific responses by anti-mouse CD4 antibody (14) infer a functional interaction between human class II molecules and mouse CD4. However, in a study using transfectant APC expressing mouse/human chimaeric class II molecules and mouse T cell clones as responders, it was shown that there was considerable functional impairment if the β 2 domain was switched from mouse to human, although this was not absolute since stimulation by APC was stronger than seen in experiments with CD4 negative mutants (36). A recent study showed that chimaeric molecules in which the β 2 domain of human transgenic class II was substituted by mouse H-2E could interact effectively with mouse T cells, although no comparison was made with the non-substituted counterpart (17). Another study showed that transgenic mice coexpressing Hu-CD4 and HLA-DR4 could respond to HA 307-319 in a non-mouse-CD4 dependent manner (37). We have made a direct comparison using titration of an HLA-restricted response to peptide in matched transgenic groups which either have or lack a species matched CD4/class II interaction. We believe that the CD4 transgene we have used, although not limited in its expression to the murine CD4 SP lineage, should have the potential to participate in repertoire selection since it is expressed on DP thymocytes. The caveat that this transgene, like the other Hu-CD4 constructs which have been used to date, does not carry all of the promoter and enhancer sequences involved in directing normal developmental control of the gene is an important one when considering functional effects. However, a recent study showed that even when the Hu-CD4 cDNA was expressed from a CD2 cassette, the presumably disregulated CD4 product was competent to rescue TCR positive selection in mice carrying a targeted disruption of murine CD4 (16). The fact that we saw no difference between Hu-CD4⁺ and Hu-CD4⁻ littermates supports the notion, implied by the similar T cell responses in HLA class II transgenic mice in separate studies with or without matched CD4 (14, 17), that for some responses at least the requirement may not be absolute. It may be neces-

sary to examine responses after weaker priming or to antigens which elicit less potent responses to identify a stronger human CD4 dependence. It is noteworthy that in mutagenesis studies done to map the interaction site between human class I and CD8, mutations which abrogated this binding had little effect at the bulk population level and were only seen to prevent function at the level of selected clones (38). An analogous effect may be operating here such that in bulk responses the proliferation of clones which are not markedly CD4-dependent masks the lack of response in others.

A key application of HLA class II transgenic mice is in the analysis of HLA-associated autoimmune disease. While we have a relatively clear understanding of the molecular interactions between TCR, MHC, and peptide which lead to specific T cell activation in experimental disease models such as experimental allergic encephalomyelitis (39), there are no human autoimmune diseases where each of these components has been elucidated in a way which would allow therapeutic intervention. In many diseases the best available handle is the genetic association with particular HLA class II alleles, although whether this reflects a simple immune response gene effect in antigen presentation of autoantigen or some more complex mechanism is not clear (40). Furthermore, it has proved impossible to identify unequivocal autoantigenic epitopes of pathogenic T cells. Among the many obstacles to this goal have been the immunogenetic heterogeneity making difficult the study of T cell responses in human disease groups. When considering the fact that for many autoantigens both patients and controls often respond to the same self-epitopes, there is no criterion for identifying a cell which is functionally capable of mediating pathology compared to one which is not. HLA transgenic mice may help in each of these areas since individual HLA alleles can be examined in isolation and clones (which could be either HLA-restricted mouse clones against autoantigenic peptides or human T cell clones transferred to transgenic mice or Hu-SCID HLA transgenic mice) can be tested using in vivo models of pathogenesis. Of the reported T cell responses to MBP by MS patients and con-

trols, there is considerable variation in the findings, some groups identifying rather limited patterns of epitope recognition and TCR usage in diseased individuals (41, 42, 43), while others have found enormous heterogeneity (18, 44). We noted however that various studies found responses to an MBP epitope in the region of 139-154 in HLA-DR1⁺ patients (18, 19). As a first step towards building "humanized" disease models in HLA transgenic mice we asked whether HLA-DR1 transgenic mice acquired responsiveness to this

epitope. It is conserved between the MBP sequences of humans and mice except for a conservative valine for alanine substitution at 147. We obtained T cell responses to this epitope in transgenic mice but not in non-transgenic controls; as yet we have seen no HLA transgene-dependent autoimmune disease in immunized mice. We are currently conducting a wider study utilizing transgenic lines expressing various disease associated alleles in the hope of making further progress in this area.

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Address correspondence to Dr. D. Altmann, Transplantation Biology, MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK. Hidetoshi Inoko's current address is Tokai University School of Medicine, Bohseidai, Isehara 259-11, Japan.

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References

1. Kievits, F., P. Ivanyi, P. Krimpenfort, A. Berns, and H. Ploegh. 1987. HLA-restricted recognition of viral antigens in HLA transgenic mice. *Nature (Lond.)* 329:447-449.
2. Kievits, F., W. Lokhorst, and P. Ivanyi. 1990. Abnormal antiviral immune response in mice is corrected in HLA-B27.2 transgenic mice. *Eur. J. Immunol.* 20:1189-1192.
3. Hammer, R.E., S.D. Maika, J.A. Richardson, J.P. Tang, and J.D. Taurog. 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β_2m : an animal model of HLA-B27-associated human disorders. *Cell* 63: 1099-1112.
4. Breban, M., R.E. Hammer, J.A. Richardson, and J.D. Taurog. 1993. Transfer of the inflammatory disease of HLA-B27 transgenic rats by bone marrow engraftment. *J. Exp. Med.* 178: 1607-1616.
5. Le, A.-X.T., E.J. Bernhard, M.J. Holterman, S. Strub, P. Parham, E. Lacy, and V.H. Engelhard. 1989. Cytotoxic T cell responses in HLA-A2.1 transgenic mice. Recognition of HLA alloantigens and utilization of HLA-A2.1 as a restriction element. *J. Immunol.* 142:1366-1371.
6. Epstein, H., R. Hardy, J.S. May, M.H. Johnson, and N. Holmes. 1989. Expression and function of HLA-A2.1 in transgenic mice. *Eur. J. Immunol.* 19:1575-1583.
7. Kalinke, U., B. Arnold, and G. Hammerling. 1990. Strong xenogeneic HLA response in transgenic mice after introducing an $\alpha 3$ domain into HLA B27. *Nature (Lond.)* 348:642-644.
8. Lawrance, S.K., L. Karlsson, J. Price, V. Quaranta, Y. Ron, J. Sprent, and P. Peterson. 1989. Transgenic HLA DRA faithfully reconstitutes IE controlled immune functions and induces cross tolerance to E α in E o mutant mice. *Cell* 58:583-594.
9. Zhou, P., G.D. Anderson, S. Savarirayan, H. Inoko, and C.S. David. 1991. Thymic deletion of V β 11⁺, V β 5⁺ T cells in H-2E negative HLA-DQ β ⁺ single transgenic mouse. *J. Immunol.* 146:854-859.
10. Fukui, Y., A. Esaki, A. Kimura, K. Hirokawa, Y. Nishimura, and T. Sasazuki. 1993. T cell repertoire in a strain of transgenic C57BL/6 mice with the HLA-DRA gene on the X-chromosome. *Immunogenetics* 37:204-211.
11. Altmann, D.M., K. Takacs, J. Trowsdale, and J.I. Elliott. 1993. Mouse mammary tumor virus-mediated T cell receptor negative selection in HLA-DRA transgenic mice. *Human Immunol.* 37:149-156.
12. Elliott, J.I., K. Takacs, and D.M. Altmann. 1993. HLA-DR and H-2E transgenes differentially mediate TCR-specific positive selection. *Int. Immunol.* 5:1279-1284.
13. Nishimura, Y., T. Iwanaga, T. Inamitsu, Y. Yanagawa, M. Yasunami, A. Kimura, K. Hirokawa, and T. Sasazuki. 1990. Expression of the human MHC HLA-DQw6 genes alters the immune response in C57BL/6 mice. *J. Immunol.* 145:353-360.
14. Yamamoto, K., Y. Fukui, T. Esaki, T. Inamitsu, K. Sudo, N. Yamane, N. Kamikawaji, A. Kimura, and T. Sasazuki. 1994. Functional interaction between human histocompatibility leukocyte antigen (HLA) class II and mouse CD4 molecule in antigen recognition by T cells in HLA-DR and DQ transgenic mice. *J. Exp. Med.* 180:165-171.
15. Schwartz, B.D. 1994. HLA class II transgenic mice: the chance to unravel the basis of HLA class II associations with disease. *J. Exp. Med.* 180:11-13.
16. Law, Y.M., R.S.M. Yeung, C. Mamalaki, D. Kioussis, T.W. Mak, and R.A. Flavell. 1994. Human CD4 restores normal T cell development and function in mice deficient in murine CD4. *J. Exp. Med.* 179:1233-1242.
17. Woods, A., H.Y. Chen, M.E. Trumbauer, A. Sirotina, R. Cummings, and D.M. Zaller. 1994. Human major histocompat-

- bility complex class II-restricted T cell responses in transgenic mice. *J. Exp. Med.* 180:173-181.
18. Pette, M., K. Fujita, D. Wilkinson, D.M. Altmann, J. Trowsdale, G. Giegerich, A. Hinkkanen, J.T. Epplen, L. Kappos, and H. Wekerle. 1990. Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple sclerosis patients and healthy donors. *Proc. Natl. Acad. Sci. USA.* 87:7968-7972.
 19. Valli, A., A. Sette, L. Kappos, C. Oseroff, J. Sidney, G. Miescher, M. Hochberger, E.D. Albert, and L. Adorini. 1993. Binding of myelin basic protein peptides to human histocompatibility leukocyte antigen class II molecules and their recognition by T cells from multiple sclerosis patients. *J. Clin. Invest.* 91:616-628.
 20. Meunier, H.F., S. Carson, W.F. Bodmer, and J. Trowsdale. 1986. An isolated $\beta 1$ Exxon next to the DR α gene in the HLA-D region. *Immunogenetics.* 23:172-181.
 21. Taketo, M., A.C. Schroeder, L.E. Mobraaten, K.B. Gunning, G. Hanten, R.R. Fox, T.H. Roderick, C.L. Stewart, F. Lilly, C.T. Hansen, and P.A. Overbeek. 1991. FVB/N: an inbred mouse strain preferable for transgenic analysis. *Proc. Natl. Acad. Sci. USA.* 88:2065-2069.
 22. Lores, P., V. Boucher, C. Mackay, M. Pla, H. von Boehmer, J. Jami, F. Barre-Sinoussi, and J.-C. Weill. 1992. Expression of human CD4 in transgenic mice does not confer sensitivity to human immunodeficiency virus infection. *AIDS Res. and Hum. Retroviruses.* 12:2063-2071.
 23. Lampson, L., and R. Levy. 1980. Two populations of IA-like molecules on a human B cell line. *J. Immunol.* 125:293-299.
 24. De Kretser, T.A., M. Crumpton, J.G. Bodmer, and W.F. Bodmer. 1982. Demonstration of two distinct light chains in HLA-DR associated antigens by two-dimensional gel electrophoresis. *Eur. J. Immunol.* 12:214-221.
 25. Maddox, J.F., and J.G. Bodmer. 1989. Hybrid human-mouse class II molecules: localisation of antibody binding sites. In *Immunobiology of HLA Vol. II, Immunogenetics and Histocompatibility*. B. Dupont, editor. Springer Publishing Co., New York. 373 pp.
 26. Altmann, D.M., J.M. Heyes, H. Ikeda, A. Sadler, A. Wilkinson, J.A. Madrigal, J.G. Bodmer, and J. Trowsdale. 1990. Fine mapping of HLA class II monoclonal antibody specificities using transfected L cells. *Immunogenetics.* 32:51-55.
 27. Pullen, A.M., P. Marrack, and J.W. Kappler. The T-cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. 1988. *Nature (Lond.)* 335:796-801.
 28. Tomonari, K., E. Lovering, and S. Spencer. 1990. Correlation between the V-Beta-4⁺ CD8⁺ T-cell population and the H-2^d haplotype. *Immunogenetics.* 31:333-337.
 29. Staerz, U.D., H.G. Rammensee, J.D. Benedetto, and M.J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3394-4000.
 30. Woodland, D.L., M.P. Happ, J. Bill, and E. Palmer. 1990. Requirement for co-tolerogenic gene products in the clonal deletion of I-E reactive T cells. *Science (Wash. DC)* 247:964-967.
 31. Zaller, D.M., G. Osman, O. Kanagawa, and L. Hood. 1990. Prevention and treatment of murine experimental allergic encephalomyelitis with T cell receptor V β -specific antibodies. *J. Exp. Med.* 171:1943-1955.
 32. Lamb, J.R., and N. Green. 1983. Analysis of the antigen specificity of influenza haemagglutinin-immune human T lymphocyte clones: identification of an immunodominant region for T cells. *Immunology.* 50:659-666.
 33. Panina-Bordignon, P., A. Tan, A. Termijtelen, S. Demotz, G. Corradin, and A. Lanzavecchia. 1989. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur. J. Immunol.* 19:2237-2242.
 34. De Magistris, M.T., M. Romano, A. Bartolini, R. Rappuoli, and A. Tagliabue. 1989. Human T cell clones define S1 subunit as the most immunogenic moiety of pertussis toxin and determine its epitope map. *J. Exp. Med.* 169:1519-1532.
 35. Inamitsu, T., Y. Nishimura, and T. Sasazuki. 1992. Different recognition of transgenic HLA-DQw6 molecules by CD4⁺ and CD8⁺ T cells. *Immunogenetics.* 35:46-50.
 36. Vignali, D.A.A., J. Moreno, D. Schiller, and G.J. Hammerling. 1992. Species-specific binding of CD4 to the $\beta 2$ domain of major histocompatibility complex class II molecules. *J. Exp. Med.* 175:925-932.
 37. Fugger, L., S.A. Michie, I. Rulifson, C.B. Lock, and G. Sonderstrup McDevitt. 1994. Expression of HLA-DR4 and human CD4 transgenes in mice determines the variable region β -chain T cell repertoire and mediates an HLA-DR restricted response. *Proc. Natl. Acad. Sci. USA.* 91:6151-6155.
 38. Salter, R.D., R.J. Benjamin, P.K. Wesley, S.E. Buxton, T.P.J. Garrett, C. Clayberger, A.M. Krensky, A.M. Norment, D.R. Littman, and P. Parham. 1990. A binding site for the T cell receptor co-receptor CD8 on the $\alpha 3$ domain of HLA-A2. *Nature (Lond.)* 345:41-46.
 39. Steinman, L., 1992. Multiple sclerosis and its animal models: the role of the major histocompatibility complex and the T cell receptor repertoire. *Springer Semin. Immunopathol.* 14:79-93.
 40. Altmann, D.M., D. Sansom, and S.G.E. Marsh. 1991. What is the basis for HLA-DQ associations with autoimmune disease? *Immunol. Today.* 12:267-270.
 41. Ota, K., M. Matsui, E. Milford, G.A. Mackin, H.L. Weiner, and D.A. Hafler. 1990. T cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature (Lond.)* 346:183-187.
 42. Oksenberg, J.R., M.A. Panzara, A.B. Begovich, D. Mitchell, H. Erlich, R.S. Murray, R. Shimonkevitz, M. Sherrit, J. Rothbard, C.C.A. Bernard, and L. Steinman. 1993. Selection for T cell receptor V β -D β -J β gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature (Lond.)* 362:68-70.
 43. Kotzin, B.L., S. Karuturi, Y.K. Chou, J. Lafferty, J.M. Forrester, M. Better, G.E. Nedwin, H. Offner, and A.A. Vandembark. 1991. Preferential T cell receptor β -chain variable gene use in myelin basic protein-reactive T cell clones from patients with multiple sclerosis. *Proc. Natl. Acad. Sci. USA.* 88:9161-9165.
 44. Ben-Nun, A., R. Liblau, L. Cohen, D. Lehmann, E. Tournier-Lasserre, A. Rosenzweig, Z. Jingwu, J. Raus, and A.M. Bach. 1991. Restricted T cell receptor V β usage by myelin basic protein specific T cell clones in multiple sclerosis: predominant genes vary in individuals. *Proc. Natl. Acad. Sci. USA.* 88:2466-2470.