Dual T Cell Receptor α Chain T Cells In Autoimmunity

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Summary

Allelic exclusion at the T cell receptor α locus *TCR-α* is incomplete, as demonstrated by the presence of a number of T lymphocyte clones carrying two expressed α chain products. Such dual α chain T cells have been proposed to play a role in autoimmunity, for example, because of a second TCR-αβ pair having bypassed negative selection by virtue of low expression. We examined this hypothesis by generating mice of various autoimmunity-prone strains carrying a hemizygous targeted disruption of the *TCR-α* locus, therefore unable to produce dual α chain T cells. Normal mice have a low but significant proportion of T cells expressing two cell-surface TCR-α chains that could be enumerated by comparison to TCR-α hemizygotes, which have none. Susceptibility to various autoimmune diseases was analyzed in TCR-α hemizygotes that had been backcrossed to disease-prone strains for several generations. The incidence of experimental allergic encephalomyelitis and of lupus is not affected by the absence of dual TCR-α cells. In contrast, nonobese diabetic (NOD) TCRα hemizygotes are significantly protected from cyclophosphamide-accelerated insulitis and diabetes. Thus, dual α T cells may play an important role in some but not all autoimmune diseases. Furthermore, since protected and susceptible NOD mice both show strong spontaneous responses to glutamic acid decarboxylase, responses to this antigen, if necessary for diabetetogenesis, are not sufficient.

It is evident from analysis of T cell clones and TCR transgenic mice that allelic exclusion is incomplete for the *TCR-α* locus, as many T cells are found to carry two functionally rearranged products (1–3). Using human peripheral blood, it was estimated from the frequency of cells giving dual staining for two different Vα chain antibodies that ~30% of human peripheral T cells may express two functional TCR-α chains (4). Such findings may have important implications for autoimmune disease: any dual TCR-α T cell may be positively selected using one TCR-αβ pair, but may prove autoreactive via its second αβ combination, particularly if escape is facilitated by the second receptor being expressed at low density on emergent cells (5). For example, in TCR transgenic mice expressing an anti–H-$2K^b$ receptor, cells expressing only the clonotypic αβ pair showed high TCR expression, while cells expressing two α chains paired with the transgenic β chain had a lower TCR density. On crossing TCR transgenic mice with a transgenic line expressing the target antigen, only the TCR^{hi} cells were deleted (5).

We have explored the potential role of dual TCR-α T cells in autoimmune disease by backcrossing mice of several autoimmunity-prone strains onto a strain carrying a TCR-α gene disrupted by homologous recombination (6). Mice homozygous for this mutation lack αβ TCR–bearing cells, but in the hemizygous state, they appear phenotypically and functionally normal (6, 7), including the number and proportion of $CD3^+$ cells in the thymus and periphery (Elliott, J., unpublished observations). To assess the effect of the hemizygous TCR-α mutation (and consequent inability to generate dual α T cells) on type I diabetes, experimental allergic encephalitis (EAE)[1], and lupus, the mutation was bred for six or more generations onto each of the strains: nonobese diabetic (NOD), SJL, and MRL lpr/lpr.

Materials and Methods

Mice. A targeted mutation of the *TCR-α* locus in 129 strain mice (6) was crossed for several generations onto NOD, SJL (CRC, Harrow, London, UK), and MRL/lpr (Olac, Bicester, UK) backgrounds. Mice carrying the targeted disruption were identified by PCR (8). Mice crossed to the NOD, SJL, and MRL/lpr strains were analyzed for disease incidence at six to seven backcross generations. Wild-type controls in each experiment are the negative littermates from mating of TCR-α hemizygotes.

Genotypic Analysis. In the case of TCR-α hemizygous mice crossed onto the NOD background, genotypic analysis was conducted to analyze the relative contributions of NOD and 129 sequences, particularly in regions near to proposed diabetes susceptibility loci. The microsatellite markers used were those described previously (9–11). Additional markers were obtained from the

[1] *Abbreviations used in this paper:* EAE, experimental allergic encephalitis; GAD, glutamic acid decarboxylase; NOD, nonobese diabetic; PLP, proteolipoprotein.

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Whitehead Institute Genome Database (Cambridge, MA). The markers were Adh-1, D3Nds5, D4Mit71 (*Idd11*), D4Mit13, Zp-3, Ly-3, Ckmm (*Idd7*), Gfap (*Idd4*), Igh, D12Nds7, D14Mit11 (*Idd12*), TCR-α, Nf1, H-2 (*Idd1*), D1Mit5 (*Idd5*), D4Nds16 (*Idd9*), D14Mit1, Qb-1, Ap2, D6Nds6, and Acrb.

Flow Cytometry. 10^6 mesenteric lymph node cells were stained at 4°C with FITC-conjugated anti-Vα8 (PharMingen, San Diego, CA) and PE-conjugated anti-Vα8 (PharMingen), and analyzed on a FACScan® (Becton Dickinson & Co., Mountain View, CA). A gate was defined such that the difference between the $V\alpha 2^+8^+$ population in wild-type mice and fluorescence background, as indicated by analysis of TCR-α hemizygote mice, was maximized. At least 100,000 cells were acquired for each sample.

Disease Induction and Analysis. To induce EAE, mice received 200 μg of proteolipoprotein (PLP) 139-151 peptide emulsified in IFA supplemented with 60 μg mycobacteria (*Mycobacterium tuberculosis* and *Mycobacterium butyricum* 8:1) subcutaneously on days 0 and 7 (12). Immediately after injection of antigen and 24 h later, mice received 200 ng of *Bordetella pertussis* toxin (Porton Products Ltd., Salisbury, UK) in PBS intraperitoneally. Mice were checked for signs of disease from day 11: 0 = normal; 1 = limp tail; 2 = impaired righting reflex; 3 = partial hindlimb paralysis; 4 = complete hindlimb paralysis; 5 = moribund.

To accelerate diabetes in mice on the NOD background, $TCR\text{-}\alpha^{+/+}$ and $TCR\text{-}\alpha^{+/-}$ mice were injected intraperitoneally with 200 mg/kg cyclophosphamide (Asta Medica, Cambridge, UK) (a drug that, while increasing the rapidity and incidence of diabetes in the NOD mouse, does not induce insulitis or diabetes in normal mouse strains [13, 14], NOD.*H-2^b* mice [15], or F_1 crosses of NOD with normal mice [14]) twice within a 14-d interval. 28 d after the first injection, the mice were killed. Blood glucose was measured at the time of death using Glucostix (Bayer Diagnostics, Basingstoke, UK). Mice were 6–6.5 mo old at time of death. They were housed in a non–specific pathogen–free facility, which retarded spontaneous disease by ~3 mo compared with incidence in our specific pathogen–free unit. Hematoxylin and eosin–stained pancreas sections were scored blind for islet pathology: 1 = peri-islet infiltration; 2 = mild intra-islet infiltration; 3 = severe intra-islet infiltration; 4 = loss of islet architecture. Normal blood glucose was defined as being less than the mean ± 2.5 SD of readings from age-matched NOD mice carrying an H-2Eα transgene, which confers complete protection from diabetic pathology (16).

To assess susceptibility to lupus, sixth generation backcross MRL lpr/lpr $TCR\alpha^{+/+}$ and $TCR\alpha^{+/-}$ mice were immunized intradermally with 0.1 ml CFA to accelerate disease (17). Day 28 serum samples were tested for α-dsDNA antibodies in an ELISA using S1 nuclease–treated calf thymus DNA (Sigma Immunochemicals, St. Louis, MO) –coated plates, essentially as described elsewhere (18). Mice were tested for hematuria on day 28, killed, and their blood was tested for uremia. Samples were assessed for hematuria using Labstix (Bayer Diagnostics) and for uremia using Azostix (Bayer Diagnostics).

T Cell Proliferation Assays. Splenocytes from untreated NOD $TCR\alpha^{+/+}$ or $TCR\alpha^{+/-}$ 12-wk-old mice were cultured in HL1 medium supplemented with antibiotics, glutamine, and 2-ME (Hycor Biomedical Inc., Irvine, CA) at 4×10^5 cells per well with 25 μg/ml recombinant rat GAD67 protein (encompassing amino acids 332–584) expressed in the pGEX1 vector or with control pGEX fusion protein (gifts from Dr. O. Birk, Weizmann Institute, Rehovot, Israel). As an additional control for any mitogenic or nonspecific effect of the antigen preparations, responses were compared between female mice and male littermates that were only mildly susceptible to disease. Assays were cultured for 72 h, pulsed with 1 μCi [^{3}H]thymidine per well for the final 6 h and harvested for liquid scintillation counting.

Results

Frequency of Dual TCR-α Cells. We compared TCR-α hemizygote (lacking dual α T cells) and wild-type littermates for the presence of T cells carrying both Vα2 and Vα8 (Fig. 1). To calculate the frequency of dual α T cells in wild-type mice (Table 1), the number of lymphocytes found in the double-positive FACS® gate above the background staining from equivalently gated $TCR\alpha^{+/-}$ littermate cells was derived. The frequency of such lymphocytes suggests that ~10% of mature T cells express two cell-surface α chains. This estimated percentage of dual α cells is derived from the observed frequency of $V\alpha 2^+$ and $V\alpha 8^+$

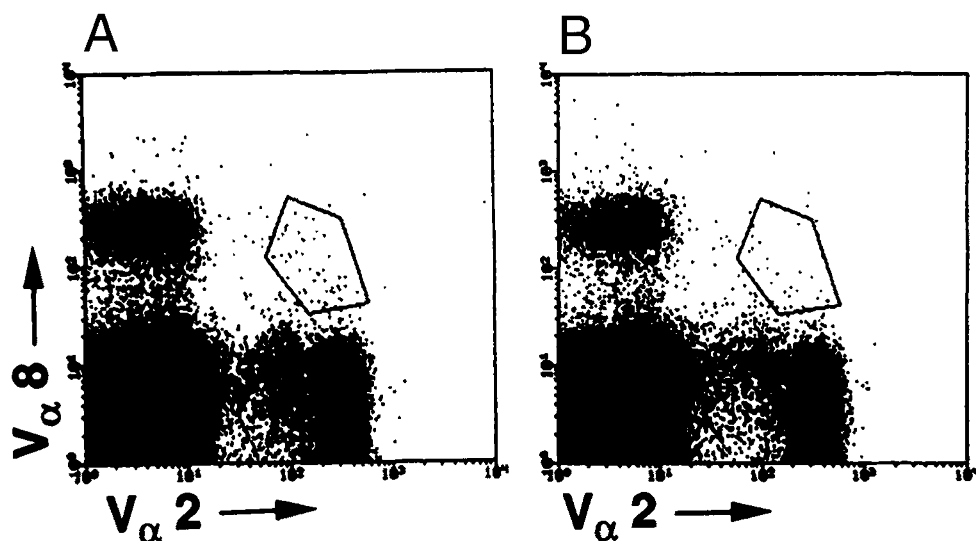

Figure 1. Identification of cells expressing two Vα chains. (*A*) Wild-type or (*B*) hemizygote littermates were compared for staining of a $V\alpha 2^+8^+$ population.

Table 1. *Frequency of Cells Expressing Two Vα Chains*

	Number per 100,000 T cells			$\alpha2^+8^+$ (Background subtracted)	Estimated Percentage of cells bearing two TCR-α chains
	$V\alpha2^+$	$V\alpha8^+$	Number of events in double-positive gate		
NOD TCR-$\alpha^{+/-}$	7,700 ± 270	2,600 ± 100	10.8 ± 3.7	0	3.700
NOD TCR-$\alpha^{+/+}$	8,300 ± 390	2,700 ± 130	35.0 ± 9.9	24.2	10.8

The frequency of T cells for $V\alpha2^+$, $V\alpha8^+$, or $V\alpha2^+8^+$ is indicated. The mean number of cells within the gated region for TCR-$\alpha^{+/-}$ mice (n = 5) was 10.8, and this was subtracted from the gated cell count of wild-type littermates (n = 5). Parallel staining of samples with FITC-conjugated α-CD3 was used to calculate the number of T cells in each test.

*See text for derivation of the percentage of dual TCR-α T cells.

cells. Thus, if all cells carried two cell-surface TCR-α chains, the proportion of $V\alpha2^+$ cells carrying $V\alpha8^+$ would be 0.027 × 0.083, i.e., 224:100,000. Since a mean of 24.2 $V\alpha2^+8^+$ cells were found, the frequency of dual α T cells is 24.2/224 = 10.8%.

Incidence of Diabetes and Insulitis in TCR-α Hemizygous NOD Mice. TCR-α hemizygous offspring were compared with wild-type littermates for incidence of cyclophosphamide-induced diabetes. Both female and male NOD TCR-$\alpha^{+/-}$ mice were significantly protected from induction of diabetes assessed by islet infiltration (Fig. 2) and blood glucose (Fig. 3). Only two male and one female TCR-$\alpha^{+/-}$ mice showed moderate islet infiltration and none displayed elevated blood glucose levels. Since it was possible that by backcrossing the homologous recombination event from chromosome 14 of the founder 129 mice onto NOD we may have selected against an NOD diabetes susceptibility locus, we conducted a PCR analysis with markers linked to the known NOD susceptibility loci (Table 2). The strongest susceptibility locus, which is within the H-2 region, is entirely NOD-derived in the mice that were analyzed. Furthermore, *Idd11* (D4Mit71), *Idd7* (Ckmm), and *Idd4* (Gfap) (19) are entirely of NOD origin in these mice. The microsatellites that have previously been used to define *Idd5* and *Idd9* were not informative in this strain combination. 17 of 18 NOD TCR-$\alpha^{+/-}$ mice described in

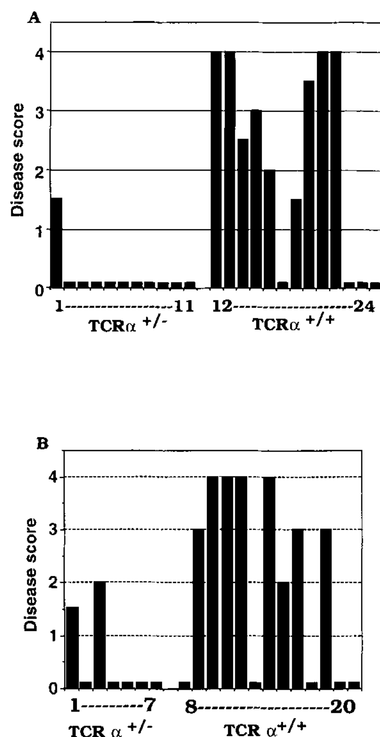

Figure 2. Incidence of insulitis in NOD TCR-$\alpha^{+/+}$ and TCR-$\alpha^{+/-}$ mice. Disease compared in (*A*) sixth generation female and (*B*) seventh generation male, NOD backcross, TCR-$\alpha^{+/+}$, and TCR-$\alpha^{+/-}$ mice.

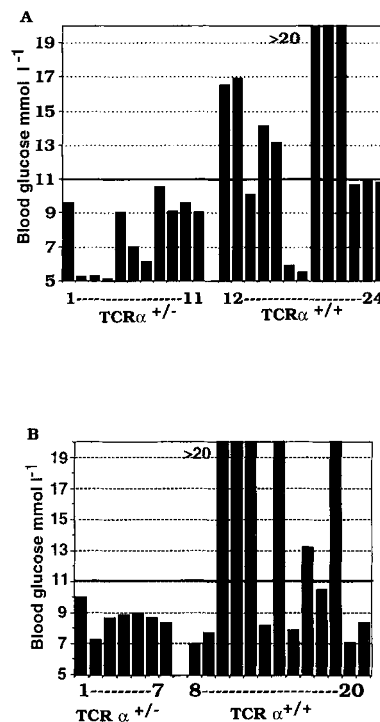

Figure 3. Incidence of diabetes in NOD TCR-$\alpha^{+/+}$ and TCR-$\alpha^{+/-}$ mice. Disease was compared in (*A*) sixth generation female, and (*B*) seventh generation male, NOD backcross, TCR-$\alpha^{+/+}$, and TCR-$\alpha^{+/-}$ mice.

Table 2. *Analysis of the Contribution from NOD and 129 Genomes in Test Mice with Respect to Microsatellite Markers Linked to Known Diabetes Susceptibility Loci*

Chromosome (location, cM)	Locus	No. mice with two copies of the NOD allele	No. mice with one copy of the 129 allele (no. displaying histopathology)
3 (83)	Adh-1	44 (20)	0
3 (86)	D3Nds5	44 (20)	0
4 (55)	D4Mit71	44 (20)	0
4 (65)	D4Mit13	44 (20)	0
5 (73)	Zp-3	44 (20)	0
6 (32)	Ly-3	19 (11)	25 (9)
7 (4)	Ckmm	44 (20)	0
11 (59)	Gfap	44 (20)	0
12 (65)	Igh	44 (20)	0
12 (66)	D12Nds7	44 (20)	0
14 (2)	D14Mit11	25 (16)	19 (4)
14 (18)	TCR-α	26 (17)	18 (3)
14 (27)	Nfl	27 (17)	17 (3)
17 (19)*	H-2	44 (20)	0
17 (19)	Qb-1	44 (20)	0

For analysis of the NOD v 129 contribution to TCR-$\alpha^{+/-}$ (n = 18) and TCR-$\alpha^{+/+}$ (n = 26) mice in the sixth and seventh backcross generation, PCR was used to distinguish between NOD and 129 microsatellites and H-2–specific markers* that were specific for H-2A^b.

this paper carry one copy of the 129 Nfl allele, located ~20 centimorgans from the TCR-α locus. Among the insulin-dependent diabetes mellitus disease markers previously mapped in a NOD backcross, the NOD alleles at two loci near the *Plau* locus on chromosome 14 conferred moderate protection (*Idd8*) in a C57BL/10 backcross (9), or moderate susceptibility (*Idd12*) in SJL and C57BL/6 backcrosses (10). While any cosegregating 129 gene would need to exert a very strong protective effect over and above any of the NOD allele, this remains a theoretical possibility, and some caution should be exercised in interpreting the results as being caused by the absence of dual TCR-α T cells until it becomes possible to repeat such studies using homologous recombination to disrupt *TCR-α* in NOD embryonic stem cells. While it is possible that the 129 contribution could decrease the overall diabetes rate, only chromosome 14 genes cosegregating with the mutant TCR-α could potentially contribute to the relative disease susceptibility between TCR-α hemizygous and wild-type mice at any given generation. While the fact that three TCR-$\alpha^{+/-}$ mice developed moderate insulitis indicates that protection which may be afforded by the absence of dual α T cells is incomplete, the results suggest that these cells may play an important role in diabetes pathogenesis.

T Cell Responses to Glutamic Acid Decarboxylase (GAD) in Protected TCR-α Hemizygous NOD Mice. Recent studies have investigated the islet autoantigens recognized by NOD T cells (20, 21); while the primary target is still unclear, GAD is a strong candidate. We compared the spontaneous T cell response to GAD 67 of NOD mice with or without dual TCR-α T cells (Fig. 4), confirming that NOD mice respond spontaneously to GAD. However, strong responses were found in both groups of mice, despite the fact that the vast majority of TCR-$\alpha^{+/-}$ mice do not develop islet pathology. The T cell response to control fusion protein in each case was in the range of 10–15% of the response to recombinant GAD protein: the mean response to control pGEX protein the experiment shown was 1,486 ± 967. As a further control for nonspecific or mitogenic effects of the GAD fusion protein, we analyzed the response to recombinant GAD in male NOD mice, which are less susceptible to disease and make reduced responses to a range of islet antigens, including GAD. We conclude that if T cell recognition of GAD is necessary for development of disease, it is not sufficient.

Incidence of EAE in Hemizygous Mutant Mice. TCR-$\alpha^{+/-}$ mice crossed onto an SJL background and wild-type littermates were compared for susceptibility to EAE induced by immunization with the dominant encephalitogenic epitope for this strain, PLP 139-151 (13). No difference was found between the two groups (Fig. 5).

Incidence of Lupus in Hemizygous Mutant MRL lpr/lpr Mice. Female backcrossed TCR-$\alpha^{+/-}$mice developed similar levels of α-dsDNA antibodies and kidney disease to wild-type littermates (Fig. 6). All mice were affected, mean peak antibodies being 0.77 ± 29 in TCR-$\alpha^{+/-}$ mice (absorbance at 1/50 dilution) and 0.82 ± 0.32 in TCR-$\alpha^{+/+}$

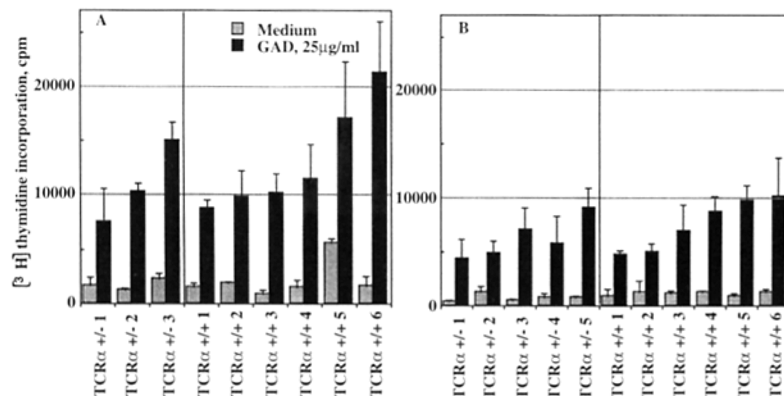

Figure 4. Both protected (*TCR*-$\alpha^{+/-}$) and susceptible (*TCR*-$\alpha^{+/+}$) NOD mice respond spontaneously to GAD. The T cell response of splenocytes from (*A*) female NOD mice and (*B*) male NOD mice in response to GAD is shown, with TCR-$\alpha^{+/-}$ mice in each case to the left of the vertical bar and TCR-$\alpha^{+/+}$ mice to the right. Black bars show the mean [^{3}H]thymidine incorporation (±SE) in triplicate cultures from individual mice.

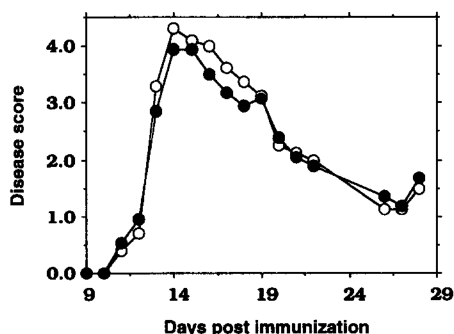

Figure 5. Both TCR-$\alpha^{+/+}$ and TCR-$\alpha^{+/-}$ mice are susceptible to EAE. Induction of EAE in SJL TCR-$\alpha^{+/+}$ (*closed circles, n* = 11) and SJL TCR-$\alpha^{+/-}$ mice (*open circles, n* = 5). PLP 139-151–immunized mice were scored blind for clinical symptoms. The figure shows a typical experiment: all mice in both groups were affected on day 14. The range of disease scores at this time point was 3.5–4.5 in hemizygous mice and 4–4.5 in wild-type littermates.

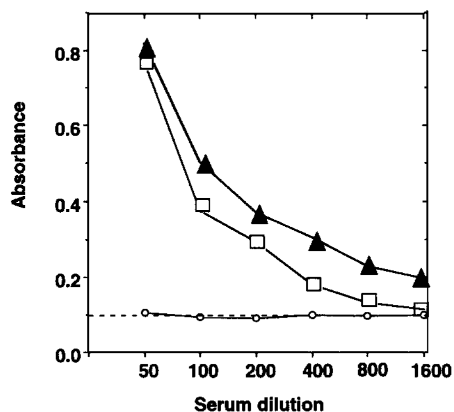

Figure 6. Both TCR-$\alpha^{+/+}$ and TCR-$\alpha^{+/-}$ mice are susceptible to lupus. α-dsDNA antibodies were measured in female MRL lpr/lpr TCR-$\alpha^{+/-}$ (*n* = 7), TCR-$\alpha^{+/+}$ littermates (*n* = 8) and C57BL/10 control mice (*n* = 6). α-dsDNA antibody ELISA readings: TCR-$\alpha^{+/-}$ (*open squares*), TCR-$\alpha^{+/+}$ (*closed triangles*), C57BL/10 (*open circles*), no serum (*dotted line*). No macroscopic evidence of arthritis was found in any mouse.

mice. No significant difference between antibody titers of TCR-$\alpha^{+/-}$ and TCR-$\alpha^{+/+}$ mice was found at any point. Analysis of hematuria and uremia also revealed no significant difference between groups (data not shown).

Discussion

The finding that a substantial proportion of human peripheral blood T cells possess two cell surface TCR-α chains and consequently two distinct TCR-αβ pairs has important implications for autoimmunity (4). It has been suggested that T cells positively selected through one αβ pair may prove autoreactive via another. We examined this hypothesis by derivation of mice from strains susceptible to autoimmune diseases and that are hemizygous for an engineered mutation in the *TCR-α* locus. Because a direct comparison can be made between FACS® staining of dual α chain cells in normal mice and hemizygotes, the true number of cells of this type can be assessed more confidently than is possible in human peripheral blood, where a figure for the proportion of dual TCR-α T cells approaching 30% was derived. A caveat to the estimate in humans is that FACS® noise (i.e., events falling within the dual a chain–positive gate as a result of nonspecific staining with antibody) may have added to the apparent frequency. The use of TCR-α hemizygous mice as a background from which to calculate FACS® noise has allowed us to show that in mice, only ~10% of peripheral T cells possess two cell-surface TCR-α chains at detectable levels, as extrapolated from the proportion of $V\alpha2^+\alpha8^+$ cells.

NOD TCR-α hemizygous mice were substantially protected from the induction of insulitis and diabetes. Importantly, such protection is not complete, as evidenced by islet infiltration in three mice in this study, as well as by the occasional occurrence of overt diabetes in TCR-α hemizygous mice during the course of backcrossing the TCR-α mutation (consequently outside of any controlled comparison with wild-type littermates). Protection in hemizygous mice may theoretically be the consequence of immunological changes and/or genetic changes at a NOD disease locus. According to the immunological explanation, which we consider the more likely, the dual TCR-α T cells would have some significant role in diabetogenesis. It is implicit in the hypothesis that the dual TCR-α T cell population is likely to harbor a disproportionately high number of autoreactive cells. Any hypothesis for the role of dual TCR-α cells in autoreactivity must explain why dual receptors are not subject to negative selection at the same stringency as single receptors. This may result if a second receptor were expressed at low density. While our data suggest that dual TCR-α T cells have a role in the pathogenesis of diabetes, it is clear that pathogenic T cells, either in this disease or in other autoimmune diseases, need not necessarily be of this type. Thus, in EAE and diabetes TCR transgenic models, the incidence of spontaneous disease actually increases when TCR transgenic mice are crossed onto a "knockout" background precluding the expression of endogenous α chains (22). Furthermore, a number of T cell clones that have been shown to transfer autoimmune disease only express a single TCR-αβ pair (23).

A more trivial explanation of protection in TCR-α hemizygote NOD would be that it is dependent on a diabetes gene linked to the TCR-α locus on chromosome 14 of the 129 strain. Since the study used wild-type littermates of TCR-α hemizygote mice as the control for diabetes incidence, the two groups differ significantly only at loci on chromosome 14, and hence it is highly unlikely that the disease susceptibility difference between the two is dependent on diabetes loci on other chromosomes. It is possible, however, that contamination with unidentified regions of the 129 genome lowered the overall diabetes incidence in both groups.

The generation of NOD mice that are resistant to the development of diabetes enabled us to ask how protection

correlated with the presence or absence of T cell responses to candidate islet autoantigens. A number of groups demonstrated that insulin-dependent diabetes mellitus patients analyzed early in disease make T cell responses to GAD (24–26). The response to the two isoforms, GAD 65 and GAD 67, are among the earliest antiislet responses detectable in NOD mice, and tolerogenic immunization with either isoform can reduce or delay the onset of disease (20, 21, 27). We analyzed responses to GAD 67 since this is the predominant GAD isoform expressed in NOD islets (28). Wild-type NOD mice, which are susceptible to diabetes, or their TCR-α hemizygous littermates, which are not, make identical responses to the GAD 67 fragment tested. This suggests that if the T cell response to GAD is indeed necessary for pathogenesis, as implied by the tolerance induction experiments, it is not sufficient. One caveat is that the fragment we used encompasses only amino acid residues, 332–584; it therefore contains most of the key epitopes that have previously described in patients or NOD mice (e.g., the 509–528 and 524–543 epitopes described by Kaufman et al. (20), the 550–585 fragment in Elliott et al. (27), and a major disease-associated epitope in patients (26). The fragment, however, lacks the 247–266 epitope recognized in some diabetes studies (20, 25).

To conclude our report, the data we present show first that dual α T cells are present in mice at a low frequency (~10%). Second, while dual α T cells are not a prerequisite for autoimmune disease per se, they may strongly predispose to the induction of NOD diabetes.

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References

1. Casanova, J.-L., P. Romero, C. Widmann, P. Kourilsky, and J.L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex–restricted cytotoxic T lymphocyte clones specific for a *Plasmodium berghei* nonapeptide: implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174:1371–1373.
2. Blüthmann, H.P. Kisielow, Y. Uematsu, M. Malissen, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz. 1988. T-cell-specific deletion of T-cell receptor transgenes allows functional rearrangement of endogenous α- and β-genes. *Nature (Lond.).* 334:156–159.
3. Malissen, M., J. Trucy, F. Letourner, N. Rebai, D.E. Dunn, F.W. Fitch, L. Hood, and B. Malissen. 1988. A T cell clone expresses two T cell receptor α genes but uses one αβ heterodimer for allorecognition and self MHC-restricted antigen recognition. *Cell.* 55:49–59.
4. Padovan, E., G. Casorati, P. Dellabona, S. Meyer, M. Brockhaus, and A. Lanzavecchia. 1993. Expression of two T cell receptor α chains: dual receptor T cells. *Science (Wash. DC).* 262:422–424.
5. Heath, W.R., and J.F.A.P. Miller. 1993. Expression of two α chains on the surface of T cells in T cell receptor transgenic mice *J. Exp. Med.* 178:1807–1811.
6. Philpott, K.L., J.L. Viney, G. Kay, S. Rastan, E.M. Gardiner, S. Chae, A.C. Hayday, and M.J. Owen. 1992. Lymphoid development in mice congenitally lacking T cell receptor αβ-expressing cells. *Science (Wash. DC).* 256:1448–1452.
7. Mombaerts, P.J., J. Arnold, H. Russ, S. Tonegawa, and S.H.E. Kaufmann. 1993. Different roles of αβ and γδ cells in immunity against an intracellular bacterial pathogen. *Nature (Lond.).* 365:53–56.
8. Picard, J.K. 1994. A PCR strategy to distinguish heterozygous and homozygous gene "knockout" transgenic mice. *Transgenics.* 1:241–243.
9. Todd, J.A., T.J. Aitman, R.J. Cornall, S. Ghosh, J.R.S. Hall, C.M. Hearne, A.M. Knight, J.M. Love, M.A. McAleer, J.-B. Prins et al. 1991. Genetic analysis of autoimmune diabetes mellitus in mice. *Nature (Lond.).* 351:542–547.
10. Morahan, G.S., P. McClive, D. Huang, P. Little, and A. Baxter. 1994. Genetic and physiological association of diabetes susceptibility with raised Na^+/H^+ exchange activity. *Proc. Natl. Acad. Sci. USA.* 91:5898–5902.
11. Love, J.M., A.M. Knight, M.A. McAleer, and J.A. Todd. 1990. Towards construction of a high resolution map of the mouse genome using PCR-analysed microsatellites. *Nucleic Acids Res.* 18, 14:4123-4127.
12. Tuohy, V.K., R.A. Sobel, Z. Lu, R.A. Laursen, and M.B. Lees. 1992. Myelin proteolipid protein: minimum sequence

requirements for active induction of autoimmune encephalomyelitis in SWR/J and SJL/J mice. *J. Neuroimmunol.* 39:67–74.

13. Harada, M., and S. Makino. 1984. Promotion of spontaneous diabetes in non-obese diabetes-prone mice by cyclophosphamide. *Diabetologia.* 27:604–606.
14. Yasunami, R., and J.-F. Bach. 1988. Anti-suppressor effect of cyclophosphamide on the development of spontaneous diabetes in NOD mice. *Eur. J. Immunol.* 18:481–484.
15. Wicker, L.S., M.C. Appel, F. Dotta, A. Pressey, B.J. Miller, N.H. DeLarato, P.A. Fischer, R.C. Boltz, and L.B. Peterson. 1992. Autoimmune syndromes in major histocompatibiity complex (MHC) congenic strains of nonobese diabetic (NOD) mice. The NOD MHC is dominant for insulitis and cyclophosphamide-induced diabetes. *J. Exp. Med.* 176:67–77.
16. Lund, T., L. O'Reilly, P. Hutchings, O. Kanagawa, E. Simpson, R. Gravely, P. Chandler, J. Dyson, J.K. Picard, A. Edwards, D. Kioussis, and A. Cooke. 1990. Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A β-chain or normal I-E chain. *Nature (Lond.).* 345:727–729.
17. Ratkay, L.G., L. Zhang, J. Tonzetich, and J.D. Waterfield. 1993. Complete Freund's adjuvant induces an earlier and more severe arthritis in MRL-lpr mice. *J. Immunol.* 151: 5081–5087.
18. Isenberg, D.A., C. Dudeney, W. Williams, I. Addison, S. Charles, J. Clarke, and A. Todd-Pokropek. 1987. Measurement of anti-DNA antibodies: a reappraisal using five different methods. *Annu. Rheum. Dis.* 46:448–456.
19. Ghosh, S., S.M. Palmer, N.R. Rodrigues, H.J. Cordell, C.M. Hearne, R.J. Cornall, J.-B. Prins, P. McShane, G.M. Lathrop, L.B. Peterson et al. 1993. Polygenic control of autoimmune diabetes in nonobese diabetic mice. *Nature Genet.* 4:404–409.
20. Kaufman, D.L., M. Clare-Salzer, J. Tian, T. Forsthuber, G.S.P. Ting, P. Robinson, M.A. Atkinson, E.E. Sercarz. A.J. Tobin, and P.V. Lehmann. 1993. Spontaneous loss of tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature (Lond.).* 366:69–72.
21. Tisch, R., X.-D. Yang, S.M. Singer, R.S. Liblau, L. Fugger, and H. McDevitt. 1993. Immune responses to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature (Lond.).* 366:72–75.
22. Lafaille, J.J., K. Nagashima, M. Katsuki, and S. Tonegawa. 1994. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell.* 78:399–408.
23. Candeias, S., J. Katz, C. Benoist, D. Mathis, and K. Haskins. 1991. Islet-specific T cell clones from non-obese diabetic mice express heterogeneous T cell receptors. *Proc. Natl. Acad. Sci. USA.* 88:6167–6170.
24. Honeyman, M.C., D.S. Cram, and L.C. Harrison. 1993. Glutamic acid decarboxylase 67–reactive T cells: a marker of insulin dependent diabetes. *J. Exp. Med.* 177:535–540.
25. Atkinson, M.A., M.A. Bowman, L. Campbell, B.L. Darrow, D.L. Kaufman, and N.K. McLaren. 1994. Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J. Clin. Invest.* 94:2125–2129.
26. Lohmann, T., R.D. Leslie, M. Hawa, M. Geysen, S. Rodda, and M. Londei. 1994. Immunodominant epitopes of glutamic acid decarboxylase 65 and 67 in insulin dependent diabetes mellitus. *Lancet.* 343:1607–1608.
27. Elliott, J.F., H-Y. Qin, S. Bhatti, D.K. Smith, R. Kumari Singh, T. Dillon, J. Lauzon, and B. Singh. 1994. Immunization with the larger isoform of mouse glutamic acid decarboxylase (GAD_{67}) prevents autoimmune diabetes in NOD mice. *Diabetes.* 43:1494–1499.
28. Kim, J., W. Richter, H. Aanstoot, Y. Shi, Q. Fu, R. Rajotte, G. Warnock, and S. Baekkeskov. 1993. Differential expression of GAD_{65} and GAD_{67} in human, rat and mouse pancreatic islets. *Diabetes.* 42:1799–1808.