Self Tolerance to T Cell Receptor \( \beta \) Sequences

By Fiorenza Falcioni,* Damir Vidovic*, E. Sally Ward,† David Bolin,* Geeta Singh,* Himanshu Shah,* Bertram Ober,† and Zoltan A. Nagy*

From the *Department of Inflammation/Autoimmune Diseases, Hoffmann-La Roche Inc., Nutley, New Jersey 07110; and the †Department of Microbiology, Cancer Immunobiology Center, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Summary

T cell tolerance to self is achieved by deletion or inactivation of clones recognizing peptides of self proteins presented by major histocompatibility complex molecules. A considerable fraction of self proteins accessible to the immune system is contributed by the system itself, for example, the receptors used for antigen recognition (antibodies and T cell receptors [TCRs]). Thus far, it has remained unclear, whether antigen receptors are subject to self tolerance, or on contrary, engage into network interactions implying immunity rather than tolerance. In this study, we demonstrate self tolerance to synthetic peptides corresponding to the first hypervariable region of the \( \beta \) 8.1 and \( \beta \) 8.2 TCR proteins. We also show that the tolerogenic synthetic peptide corresponds to a fragment produced by processing of the \( \beta \) protein, and conversely, that a \( \beta \) peptide not produced by processing is also not subject to self tolerance. Thus, the rules of tolerance seem to apply to antigen receptors, at least to their germline-encoded portions, in a similar fashion as to other self proteins. This finding has important implications for studies of natural and artificially induced immune networks.

Materials and Methods

Mice and Immunizations. Mice were obtained from The Jackson Laboratories (Bar Harbor, ME), except strain B10.Q-\( \beta \) (obtained from Dr. C. David, Mayo Clinic, Rochester, MN). F1 hybrids were bred in our facilities. Mice, >8 wk of age, were injected with 35 nmol of peptide antigen in CFA in the hind footpads and tailbase.

Peptides. Peptides were prepared by solid-phase synthesis and purified by reverse-phase HPLC; their homogeneity was confirmed by analytical HPLC, amino acid analysis, and fast atom bombardment mass spectroscopy.

Soluble Single-chain TCR. The TCR \( \alpha \) and \( \beta \) genes were isolated from T hybridoma 1934.4 (11) by PCR using oligonucleotides as described (12). The V genes were cloned into plasmid vectors designed for expression and secretion of immunoglobulin domains by \( \text{E. coli} \) (13). For the single-chain construct, the COOH terminus of \( \alpha \) was joined to the N\(_{\text{H2}} \) terminus of \( \beta \) by a (Gly\(_4 \)-Ser\(_3 \)) linker. A COOH-terminal His\(_6 \) tag was inserted to allow affinity purification on Ni\(^{2+}\)-NTA agarose columns. The strategy for construction of plasmids with single \( \alpha \) genes and joined \( \alpha \)+\( \beta \) genes was described previously (14). The single chain TCR construct was secreted by recombinant \( \text{E. coli} \) at 0.5–1 mg/liter, and the \( \alpha \) construct at 2 mg/liter (the \( \beta \) construct alone was poorly secreted; references 12, 14). Circular dichroism analyses of the \( \beta \) protein. We report here self tolerance to sequences corresponding to the first hypervariable region of the \( \beta \) 8.1 and \( \beta \) 8.2 proteins.
secreted molecules indicate that they are folded into β-pleated sheet structures similar to those of immunoglobulin V domains (14). For stimulation of T cells, the soluble TCR constructs were used at 2-8 μM final concentration (corresponding to 50-200 μg/ml of single chain VαVβ).

T Cell Lines, Clones, and Proliferation Assays. 9 d after immunization, the regional lymph nodes were removed aseptically, and single cell suspensions were prepared. Cells (2.5 × 10⁷ per well) were cultured in HL-1 medium (Ventrex, Portland, Maine) in flat bottom 96-well microtiter plates with or without peptide antigen for 3 d. Proliferation was measured by [3H]TdR incorporation (1 μCi per well) in triplicates during the last 16 h of culture. Cell lines were generated by culturing T cells (5 × 10⁶/ml) in RPMI 1640 medium with 10% FCS and 3.5 μM of peptide for 1 wk, and restimulating weekly with peptide, APC (2.5 × 10⁶ cells per ml), and 2 ng/ml of recombinant IL-2. The stable lines were cloned by limiting dilution as described previously (15). Proliferative response was tested by culturing T cells from lines (5 × 10⁵ per well) or cloned T cells (2 × 10⁴ per well), and syngeneic irradiated spleen cells (5 × 10⁵ per well) as APC in RPMI 1640 plus 5% FCS or human AB serum, with or without different concentrations of antigen for 3 d. Incorporation of [3H]TdR was measured during the last 6 h of culture.

Results

Pattern of T Cell Responsiveness to Peptide Vβ 8.1/18-31. We have selected several 14-amino acid–long sequences that are unique to Vβ proteins not expressed in certain mouse strains caused by genomic deletion or somatic deletion of the corresponding T cells by endogenous superantigens (1, 2, 10). The corresponding synthetic peptides (mostly from Vβ 6 and Vβ 8.1) were tested for immunogenicity in mouse strains expressing or deleting the respective TCR Vβ. We have identified one peptide from the first hypervariable region of Vβ 8.1, peptide 8.1/18-31, which induced a T cell response in H-2k mice. The pattern of responsiveness to this peptide is shown in Fig. 1. Of the five H-2k strains tested, only C57BR mounted a T cell response to 8.1/18-31. It should be noted that this is a strain with Vβ8.1 deleted from the germ line (10). The remaining strains (AKR, B10.BR, CBA, C3H), as well as all (responder × nonresponder) F1 hybrids were nonresponders (i.e., responsiveness was recessive). T cell lines from the nonresponder strains remained nonresponder after in vitro restimulation with antigen and IL-2 (data not shown), suggesting that 8.1/18-31–specific T cell precursors might have been absent. The response of C57BR T cells to 8.1/18-31 was not decreased upon mixing with C3H (nonresponder) T cells primed with the same peptide, indicating that suppression was not involved in nonresponsiveness (data not shown). We also tested representative strains of the AKXL recombinant inbred series. The progenitor strains of AKXL are AKR carrying H-2k on chromosome 17 and a full array of Vβ genes on chromosome 6, as well as C57L that is H-2h and carries the Vβ4 allele on chromosome 6 (i.e., 50% of Vβ genes including Vβ8 deleted; references 10, 16). According to the data in Table 1, the response clearly segregated with the presence of H-2k, and the absence of Vβ 8.1 expression in these strains. To address the role of TCR more directly in the observed nonresponsiveness, we tested a TCR+/β-deficient strain expressing no orb TCR at all and a TCR-/β-deficient strain expressing no orb TCR at all (10), in comparison to the wild-type strains of origin. These strains were crossed with C57BR to provide H-2k required for the response. The data demonstrated that responses occurred only in the Vβ4 congenic and the TCR–β-deficient strain but not in the wild-type controls (Table 1). Collectively, the data have demonstrated that the mechanism un-
Table 1. Response Pattern of AKXL Recombinant Inbred Strains, TCR Vβ Congeneic, and TCR Vβ-deficient Strains to Peptide Vβ 8.1/18-31

| Strain          | H-2 | Vβ 8.1 + 8.2 expression | Δcpm range* | S.I. range* | n  |
|-----------------|-----|-------------------------|-------------|-------------|----|
| AKXL-6          | k   | -                       | 5,632-25,590| 4.0-51.0    | 5  |
| AKXL-8          | k   | -                       | 3,522-13,400| 3.2-4.3     | 4  |
| AKXL-38         | k   | -                       | 10,235-20,847| 3.1-18.0   | 6  |
| AKXL-13         | k   | +                       | 16-10,580   | 1.1-2.0     | 7  |
| AKXL-21         | k   | +                       | 289-7,622   | 1.2-1.9     | 6  |
| AKXL-7          | b   | -                       | 156-2,418   | 1.0-1.2     | 3  |
| C57L            | b   | -                       | 363-3,156   | 1.0-1.2     | 3  |
| (B10.Q/Vβ~ x C57BR)F1 | (q x k) | -                       | 60,842-104,361 | 4.8-26.0  | 4  |
| (B10.Q × C57BR)F1 | (q x k) | +                       | 1,679-17,312 | 1.1-2.0   | 5  |
| (TCR/Vβ~ x C57BR)F1 | (b x k) | -                       | 27,651-144,900 | 3.7-22.0 | 5  |
| (C57BL/6 × C57BR)F1 | (b x k) | +                       | 60-16,744   | 1.0-2.4     | 5  |

* Highest and lowest secondary in vitro proliferative response of lymph node cells from the number of immunized animals (n) tested after 10 d of stimulation with 5 μM of 8.1/18-31 and 2 ng/ml of rIL-2 at antigen concentrations from 2.5 to 10 μM.

† Tested by FACS® analysis (Becton Dickinson and Co., Mountain View, CA) of spleen cells using the VB 8.1 + 8.2-specific mAb KJ 16.

Table 2. Cross-reactivity of Vfl 8.1/18-31-specific T Cells with Vβ 8.2/18-31

| Peptide in culture | Sequence     | Proliferative response (Δ/cpm/S.I.) |
|-------------------|--------------|-----------------------------------|
|                   |              | Line 1†                           | Line 2†                           |
| Vβ 8.1/18-31      | KVTLSCHQTNNHDY| 29662/4.8                          | 128157/7.8                         |
| Vβ 8.2/18-31      | KVTLSCHNQTNHNN| 83889/11.7                         | 337551/19.0                        |
| HEL/46-61         | NTDGSTDYGILQ1NSR| -988/0.9                          | -6084/0.7                          |

* Used at 19 μM.
† T cell lines were derived from 8.1/18-31 immune C57BR mice. Nine lines were altogether tested with the same results.
Table 3. **MHC Restriction of 8.1/18-31-specific T Cell Clones**

| Strain          | H-2 loci       | Proliferative response (Δcpm/S.I.) by clones* |
|-----------------|----------------|---------------------------------------------|
|                 | K A E D        | No. 3                                      |
|                 |                | No. 46                                     |
|                 |                | No. 22                                     |
|                 |                | No. 67                                     |
| C57BR           | k k k k        | 234519/2174                                |
|                 |                | 53875/196                                  |
|                 |                | 103703/382                                 |
|                 |                | 117879/614                                 |
| B10.A(4R)       | k k b b        | 243368/526                                 |
|                 |                | 32143/83                                   |
|                 |                | 10724/40                                   |
|                 |                | 65913/388                                  |
| B10.AQR         | q k d d        | 221479/669                                 |
|                 |                | 34766/144                                  |
|                 |                | nt                                        |
|                 |                | nt                                        |
| B10.T(6R)       | q q q d        | 1208/5                                    |
|                 |                | 646/5                                     |
|                 |                | nt                                        |
|                 |                | nt                                        |
| A.TL            | s k d d        | nt                                        |
|                 |                | nt                                        |
|                 |                | 22984/144                                 |
|                 |                | 56469/317                                 |
| A.TH            | s s s d        | nt                                        |
|                 |                | 237/1.7                                   |
|                 |                | 111/0.8                                   |

* Clones 3, 46, and 22 were derived from strain AKXL-38 and clone 67 from C57BR mice. Using a panel of anti-Vα and anti-Bβ antibodies (specific for Vα 2,3,2,8,11, and Vβ 2,3,4,5,1 + 5,2,6,7,8,1 + 8,2,9,10,11,12,13,14,17a), clone 3 typed Vβ7 Vα-blank, clone 22 Vβ14 Vα-blank, and clones 46 and 67 Vα-Vβ-blank by immunofluorescence. All clones were TCtL+*, CD4+, and CD8-.

Table 4. **Reactivity of Vβ Peptide-specific T Cells to a Soluble TCR Construct**

| Antigen*       | Proliferative response (Δcpm/S.I.)† |
|----------------|-------------------------------------|
|                | Clone 46†                           |
|                | Line 3¶                            |
|                | Line D¶                            |
|                | Line 4¶                            |
| 8.1/18-31      | AKXL-38                             |
| 8.1/18-31      | C57BR                              |
| 8.1/15-32      | C57BR                              |
| 8.1/15-32      | C3H                                |
| sol.Vα4/Vβ8.2  | 36204/178                           |
| sol.Vα4        | 394/2.0                             |
| 8.1/18-31      | 53875/196                           |
| 8.2/18-31      | 77663/282                           |
| 8.1/15-32      | 34733/127                           |

* Proliferative response to 5 μM of peptide and 8 μM of soluble TCR construct is shown.
† In the presence of irradiated C57BR splenic APC.
‡ The specificity and strain of origin of T cell lines are as indicated.
§ Two cell lines were tested with the same results.
** Peptide 8.1/15-32 has an NH2-terminal Thr-Gly-Gly and a COOH-terminal Met addition to 8.1/18-31.
peptide of the same TCR region, Vβ 8.1/15-32. As shown in Table 4, both C57BR mice and C3H mice (the latter nonresponder to 8.1/18-31) were able to respond to 8.1/15-32. However, T cells recognizing the extended peptide failed to respond to either the short peptide or the soluble TCR construct. Thus, the extended peptide was probably not produced by processing, and consequently, was not subject to self tolerance. These results provide direct support to the hypothesis that tolerance to the Vβ 8.1 and Vβ 8.2 proteins is the cause of nonresponsiveness to the 18-31 Vβ peptides in strains expressing these proteins as self.

Discussion

The results presented herein have demonstrated self tolerance to a germline-encoded variable portion of αβ TCRs. The detection of tolerance seemed to depend on the identity of the probing synthetic TCR peptide with the one produced from processing the relevant TCR protein. Probably because of this requirement, tolerance to synthetic TCR peptides has not been demonstrated thus far, but several laboratories were able to show T cell responses to long (usually 20mer) synthetic TCR peptides (9, 21–24). Our finding, if generalizable, demonstrates that antigen receptors, with the possible exception of their somatically diversified portions (i.e., somatic mutations in antibodies, and the third hypervariable region of TCR), are subject to tolerance, similarly to other self proteins accessible to the immune system. The somatically diversified sequences of antigen receptors may escape self tolerance, because they may not attain the concentration required for tolerance induction (25). Thus, potential network interactions could only be directed to such sequences, provided that they are presented by the individual’s MHC molecules.

These results also have implications for the therapeutic use of synthetic TCR peptides in autoimmune disease models (9, 21, 22). The reported beneficial effect of TCR peptides in autoimmune disease models has been explained by the induction of a downregulatory immune response against T cells that express the relevant receptor and are involved in autoimmune pathology (9). Our present results suggest that a T cell response to a TCR peptide can only occur when the immunizing peptide is not identical with a naturally processed tolerogenic TCR fragment. Consequently, a treatment with synthetic TCR peptides can have different outcomes. First, when the synthetic peptide corresponds to a tolerogenic fragment, no response and no therapeutic effect will ensue. Second, when the fragment corresponding to the immunogen is not produced at all by processing, a response will result, but it is likely to remain without therapeutic consequence. Indeed, this possibility has been demonstrated experimentally by Sun (26). Third, when the fragment is produced in subtolerogenic amounts by processing, the corresponding synthetic peptide will induce an immune response that could affect the cells naturally presenting this sequence, in the first place, the T cells expressing the appropriate receptor. When the latter cells are involved in autoimmune pathology, a response to them could modify the disease in two possible ways: downregulation with a beneficial effect (9, 22), or upregulation leading to exacerbation (27). Future research will have to identify ways to direct the anti-TCR response toward beneficial effects.

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Address correspondence to Dr. Zoltan A. Nagy, Department of Inflammation/Autoimmune Diseases, Hoffmann-La Roche Inc., Building 86/Room 530, 340 Kingsland Street, Nutley, NJ 07110-1199.

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