Antigen-specific T cells recognize antigen in association with class I or class II products of the MHC. T cell activation results from the formation of a ternary complex involving nominal antigen, the appropriate MHC molecule, and the TCR. Studies of the genetic structure of the TCR α and β chains have not provided a mechanism for the corecognition of antigen and MHC by the TCR (1). Research on this problem has therefore focused on two main areas; the specificity of the interaction between MHC molecules and nominal antigen, and the analysis of T cell receptors specific for well-defined epitopes.

The interaction between MHC molecules and nominal antigen has been demonstrated experimentally (2–4), but the structure/function relationship of the α and β chains of the TCR within the ternary complex has not yet been elucidated. One possibility is that one chain of the TCR is primarily responsible for binding to the MHC restricting molecule, while the other confers specificity for nominal antigen. The simplest version of this model, in which TCR α and β chains from T cells of unrelated specificity would be independently assorted to produce new MHC/antigen specificities, has been disproved by Kappler et al. (5). However, there is some evidence from the cytochrome c system that TCR α and β chains may be involved in the recognition of antigen and MHC, respectively. Fink et al. (6) have suggested that changes in the TCR β chain appear to alter the MHC restriction of some cytochrome c-reactive T cell hybridomas. More recently, Winoto et al. (7) indicated that a particular TCR α chain was expressed in the majority of cytochrome c–specific T cell lines, regardless of the strain of origin of these lines, and suggested that there was a correlation between TCR α chain usage and antigen recognition.

We have recently generated a series of T cell clones from individual DBA/2 mice, specific for sperm whale myoglobin (SpW Mb), the antigen fine specificity of which has been extensively characterized using synthetic peptides.1,2 We have studied this panel of T cell clones for their TCR β chain usage by FACS analysis using mAbs specific for a particular family of TCR Vβ chain genes.

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1 Livingstone, A. M., H. Levy, J. B. Rothbard, and C. G. Fathman. Fine specificity of two epitopes on sperm whale myoglobin. Manuscript submitted for publication.
2 Livingstone, A. M., J. B. Rothbard, and C. G. Fathman. Heterogeneity of the T cell response to sperm whale myoglobin: A clonal analysis in individual DBA/2 mice.

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Materials and Methods

Animals. DBA/2 J mice 6–8 wks old were obtained from The Jackson Laboratories, Bar Harbor, ME.

Monoclonal Antibodies. F23.1, a mouse IgG reactive with the V_β8 family of proteins was a kind gift of Dr. Mike Bevan (8). KJ16, a rat IgG that reacts with the same family of V_β as F23.1, was a kind gift of Dr. Osami Kanagawa (9). 10.2.16, a mouse IgG reactive with I-A^d, was obtained from the American Type Culture Collection (Rockville, MD), and used as a negative control (10). 8C5, a rat IgG reactive with cells of the granulocyte/macrophage lineage, was kindly provided by Dr. Robert Coffman (DNAX Research Institute, Palo Alto, CA) and was used as a negative control.

Generation and Analysis of SpW Mb-reactive T Cell Clones. The generation and characterization of the T cell clones used in this study have been described in detail elsewhere (9). Briefly, T cell lines were established in vitro from individual DBA/2 (H-2^d) mice that had been immunized with SpW Mb. After several restimulations in vitro, first with whole SpW Mb and then with the SpW Mb middle CNBr fragment (residues 56–131), the lines were cloned by limiting dilution. Clones were maintained by restimulation every 10–14 d with 5 μM SpW Mb presented by irradiated (3,300 rad) syngeneic spleen cells. The MHC restriction of the clones was determined using irradiated spleen cells from a panel of inbred mouse strains as APC. The antigen fine specificity was analyzed by assaying the clones on sperm whale and horse myoglobins, on the SpW Mb fragment 56–131, and on two sets of overlapping synthetic peptides spanning SpW Mb residues 63–84 and 100–124, respectively.

Immunofluorescence Staining. 12–14 d after restimulation, T cell clones were stained, using standard techniques, with the antibodies F23.1 and KJ16, 8C5, 10.2.16, and AT83 (11). The cells were analyzed on a FACS IV (Becton Dickinson Immunocytometry Systems, Mountain View, CA) and the results are expressed on a logarithmic scale.

Results and Discussion

The specificity of the SpW Mb-reactive DBA/2 T cell clones used in this study will be described in detail elsewhere. These clones were isolated from T cell lines that had been selected for reactivity against the middle CNBr fragment (residues 56–131) of SpW Mb, since three T cell epitopes have been identified within this fragment (12). Each line was derived from a different mouse; the first number of a clone identifies the mouse from which the clone was isolated. The clones can be broadly classified into three groups, on the basis of their reactivity to SpW and horse Mb, and to overlapping sets of synthetic peptides spanning known T cell epitopes on SpW Mb. In many cases, multiple clones with apparently identical specificity were isolated from one line, and were probably sister clones. In such instances, one representative clone was chosen for analysis.

The first group consists of six I-E^d-restricted clones, derived from five mice, specific for epitopes within the antigenic region 110–121. Four of these clones (8.2, 9.4, 11.3, and 12.2) appear to have identical specificity. However, the other two clones (14.12 and 14.13) differ in fine specificity from these four clones and from each other (see below). The second group includes five clones, isolated from five mice, the precise specificity of which has not yet been determined. They respond to the SpW Mb fragment 56–131, but not to horse Mb, nor to any of the synthetic peptides spanning residues 63–84 or 100–124. These clones (7.1, 8.3, 11.14, 12.1, and 13.26) are restricted by I-E^d. The third group is represented by three clones, from three mice, specific for an immunodominant region first described by Berkower et al. (12), involving residue 109. While all three of these clones (7.3, 11.12, and 13.12) see the same antigenic region
TABLE I

**Vβ Segment Usage in a Series of T Cell Clones, from DBA/2 Mice Recognizing SpW Mb**

| Clone* | Cells staining with:† | Antigen specificity | Restriction |
|--------|----------------------|---------------------|-------------|
|        | F23.1    | KJ16                |             |
| 8.2    | +        | +                   | 112-118     | I-E<sup>d</sup> |
| 9.4    | +        | +                   | 112-118     | I-E<sup>d</sup> |
| 11.3   | +        | +                   | 112-118     | I-E<sup>d</sup> |
| 12.2   | +        | +                   | 112-118     | I-E<sup>d</sup> |
| 14.12  | +        | +                   | 112-118<sup>§</sup> | I-E<sup>d</sup> |
| 14.13  | +        | +                   | 112-117     | I-E<sup>d</sup> |
| 8.3    | +        | -                   | ND<sup>§</sup> | I-E<sup>d</sup> |
| 11.14  | +        | +                   | ND          | I-E<sup>d</sup> |
| 12.1   | +        | +                   | ND          | I-E<sup>d</sup> |
| 13.26  | +        | -                   | ND          | I-E<sup>d</sup> |
| 7.1    | -        | -                   | ND          | I-E<sup>d</sup> |
| 7.5    | +        | -                   | 106-118     | I-A<sup>d</sup> |
| 11.12  | -        | -                   | 106-118     | I-A<sup>d</sup> |
| 13.12  | -        | -                   | 106-118     | I-A<sup>d</sup> |

* The first number identifies the mouse from which the clones were derived; thus, clones 11.3, 11.12, and 11.14 all came from mouse 11.

† T cell clones were stained as described in Materials and Methods. A + indicates a significant shift of the mean fluorescence from that obtained with an isotype-matched control antibody.

§ Clone 14.12 differs in fine specificity from the other four 112-118-specific clones (see text).

These clones respond to SpW Mb 56-131, but not to any of the peptides spanning residues 63-84 or 100-124.

(residues 106–118), they differ strikingly both in the pattern of response to synthetic peptides spanning this region, and in their crossreactivity on allogeneic stimulator cells in the absence of antigen; all are restricted by I-A<sup>d</sup>.

As a first step towards identifying the TCR genes expressed by these clones, we asked whether they were recognized by two monoclonal antibodies, F23.1 and KJ16, which are specific for the TCR V<sub>β</sub>8 gene family, KJ16 is known to bind to T cells that express a TCR V<sub>β</sub> gene segment from two of the three members of the V<sub>β</sub>8 gene family; F23.1 reacts with all three members of the V<sub>β</sub>8 TCR gene family (8, 9, 13). Cells that stain positively with F23.1 but are negative with KJ16 have recently been shown by Behlke et al. to express the V<sub>β</sub> 8.3 gene segment (16). Cells that are recognized by both antibodies could be using one of two V<sub>β</sub> gene segments (V<sub>β</sub> 8.1 or V<sub>β</sub> 8.2) known to bear the KJ16 epitope.

The results of immunofluorescence staining of these clones using F23.1 and KJ16 are shown in Table I. All six of the I-E<sup>d</sup>-restricted clones specific for epitopes with residues 110–121 stained positively with both F23.1 and KJ16. Four of the I-E<sup>d</sup>-restricted clones specific for unidentified epitopes within residues 56–131 were also positive for F23.1, two of these clones were positive for KJ16. In contrast, only one of the three I-A<sup>d</sup>-restricted clones was positive for F23.1, this clone was negative for KJ16. Thus 10 of the 11 I-E<sup>d</sup>-restricted SpW Mb–specific clones were F23.1<sup>+</sup>, whereas only one of the I-A<sup>d</sup>-restricted clones was F23.1<sup>+</sup>. Representative patterns of staining are shown in Fig. 1.

As demonstrated by the data in Table I, 10 of the I-E<sup>d</sup>-restricted T cell clones tested expressed TCR V<sub>β</sub> gene segments from the V<sub>β</sub>8 family, regardless of
epitope specificity. This predominant use of V\_k8 gene segments for I-E\^d-restricted clones cannot be explained by the recognition of one immunodominant epitope, since these 10 clones recognize at least two completely different antigenic regions within residues 56–131 of SpW Mb. Moreover, they can be further subdivided into at least five categories, based either on antigen fine specificity\(^2\) or on TCR V\_k chain usage. Of the six clones specific for epitopes within residues 110–121, four (8.2, 9.4, 11.3, and 12.2) have not yet been distinguished from one another. The epitope seen by these clones involves residues 112–118, and they respond equally well to SpW and horse Mb, although these two Mb differ at position 118. Molecular analysis of the TCR of these clones is in progress, and the same
member of the \( V_{\delta 8} \) family as well as the same \( J_{\delta} \) and \( C_{\delta} \) gene segment is expressed by all four clones.\(^3\) Clone 14.12 shows exactly the same pattern of reactivity to synthetic peptides as do the four clones described above; it responds poorly, however, to horse Mb, suggesting that the arginine-lysine substitution at position 118 has a deleterious effect on antigenicity for this clone. Clone 14.13, unlike the five clones just described, can be stimulated by peptide 102–117. The five I-E\(^d\)-restricted T cell clones, the epitopes of which have not been identified (Table I) can also be divided into three groups according to their staining patterns, since at least two different members of the \( V_{\delta 8} \) gene family are expressed in these five clones.

The three I-A\(^d\)-restricted T cell clones in this panel are also diverse in antigen specificity. All three clones specific for the antigenic region 106–118 show completely different patterns of response to peptides spanning this region. In addition, clone 11.12 crossreacts on allogeneic stimulators in the absence of antigen. Only one of these four clones (7.3) uses a member of the \( V_{\delta 8} \) family (presumably \( V_{\delta} 8.3 \)) (14). At this point, we have no information about the TCR \( V_{\delta} \) genes expressed by the other three clones in this group.

In conclusion, FACS analysis of this panel of clones, using the two monoclonal antibodies KJ16 and F23.1, demonstrated that 10 of 11 I-E\(^d\)-restricted clones from DBA/2 mice immunized with SpW Mb expressed TCR \( V_{\delta} \) genes from the \( V_{\delta 8} \) family irrespective of the epitopes of SpW Mb recognized. These data are in agreement with recent reports from Fink et al. (6), which suggest a correlation between the use of the TCR \( \beta \) chain and MHC restriction. It is clear from our data (Table I) and published data (9, 13) that the TCR \( V_{\delta 8} \) family is not used exclusively by I-E\(^d\)-restricted T cells. Thus, no simple model of TCR \( \beta \) chain selection and MHC restriction can be proposed. Sequence analysis of the TCR genes of this panel of clones, as well as transfection studies using isolated TCR \( \alpha \) and \( \beta \) chain genes, should provide important insights into the relationship between TCR \( \beta \) or \( \alpha \) chain expression and MHC restriction or epitope recognition. These studies are in progress. The MHC restricted antigen recognition of a given TCR may well be the result of combinatorial interactions between the \( \alpha \) and \( \beta \) chains, but the evidence available to date does suggest that, within a given antigen/MHC system, selection of a particular TCR \( \beta \) chain may play an important role in the MHC restriction of that response.

Summary

We have studied a panel of DBA/2 T cell clones specific for sperm whale myoglobin (SpW Mb) for TCR (T cell receptor) \( \beta \) chain gene expression by FACS analysis using the monoclonal antibodies F23.1 and KJ16 specific for the \( V_{\delta 8} \) family of the TCR \( \beta \) chain genes. Within any given specificity group, all the clones tested came from different mice. 10 of 11 I-E\(^d\)-restricted SpW Mb–specific T cell clones were F23.1\(^+\); 8 of these were also KJ16\(^+\). Only one of the three I-A\(^d\)-restricted clones tested was F23.1\(^+\); this clone was KJ16 negative. This study has demonstrated that I-E\(^d\)-restricted T cell clones from DBA/2 mice express members of the TCR \( V_{\delta 8} \) family irrespective of the epitopes of SpW Mb

\(^3\) Ishihara, T., A. M. Livingstone, and C. G. Fathman. Molecular analysis of T cell receptor gene rearrangements among murine T cell clones matched for antigen recognition and MHC restriction.
recognized. These data suggest an apparent correlation between TCR \( V_{\alpha} \) expression and MHC restriction.

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