ANTIGEN-REACTIVE T CELL CLONES RESTRICTED BY MIXED ISOTYPE A^d/E^d CLASS II MOLECULES

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The recognition of foreign antigen by CD4+ T lymphocytes is known to be restricted by heterodimeric cell surface glycoproteins consisting of α and β chains called class II (Ia) molecules (reviewed in reference 1). There exist two isotypically related murine class II molecules, namely I-A and I-E. Thus, I-A molecules consist of Aβ and Aα chains, while I-E molecules consist of Eβ and Eα chains. The individual T lymphocyte clone recognizes antigen in the context of I-A or I-E molecules. In F1 heterozygous mice, not only cis-located, but also certain allelic combinations of trans-located α and β chains within each isotype freely associate to form I-A and I-E molecules and are expressed on the cell surface to function as restriction elements (2, 3). Such F1-specific restriction elements are assumed to expand T cell specificity repertoire in F1 heterozygotes.

Recent serological and biochemical studies suggest the existence of mixed isotype class II molecules (AβEα in mice [4–9] and DRαDQα in humans [10, 11]). However, T lymphocyte clones that use such mixed isotype class II molecules as restriction elements have not been reported. Our previous studies suggested the existence of alloreactive T cell clones that recognize AβEα molecules expressed on Eα gene-introduced B57BL/6 (B6Eα) transgenic mouse spleen cells (12). We speculated that such AβEα molecules in B6Eα transgenic mice are created by the presence of large amounts of free Eα molecules (13) that associate with endogenous Aβ molecules by overcoming low pairing efficiency. In this report, we describe the existence of keyhole limpet hemocyanin (KLH)-specific T cell clones derived from (BALB/c × B6Eα)F1 mice that recognize AβEα molecules as restriction elements. The existence of such mixed isotype class II-restricted T cell clones would provide important implications for the expansion of T cell repertoire as well as the induction of autoimmune phenomenon.

Materials and Methods

Mice. The original Eα gene-introduced C57BL/6 (B6Eα) transgenic mice (14) were obtained from Dr. T. Kishimoto (Osaka University, Osaka, Japan) and bred to homozygous for Eα transgenes in our animal breeding facilities. BALB/c, C37BL/6 (B6), and (BALB/c x B6Eα)F1 were maintained in our animal facilities.

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Antigen-specific T Cell Clones. KLH was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Derivation of KLH-reactive T cell clones was performed essentially as described previously (3). Cloning was performed by limiting dilution at a cell density of 3 cells/well, which was repeated several times. 10^4 T cell clones were cultured with 50 µg/ml of KLH in the presence of irradiated (3,300 rad) spleen cells as APCs for 2 d. Proliferative responses of T cell clones were measured by the uptake of [3H]TdR and expressed as a mean cpm of triplicate cultures ± SD.

mAbs. The origin and specificities of mAbs used in this paper are described in Table II. Culture supernatants of each hybridoma cell line were 10-fold concentrated by 50% ammonium sulfate precipitation, dialyzed against PBS (pH 7.2), and used for mAb blocking experiments.

Results

KLH-specific T Cell Lines and Clones Derived from (BALB/c × B6E6)F1 Mice. KLH-reactive T cell lines and clones were obtained from (BALB/c × B6E6)F1 mice. The proliferative responses against KLH in the presence of (BALB/c × B6E6)F1 or CBF1 APCs are shown in Table I. Almost all the clones showed a similar degree of the KLH-specific proliferative responses in the presence of (BALB/c × B6E6)F1 and CBF1 APCs as represented by clones KTF1.1 and KTF2.1. Clones KTF1.2 and KTF2.7, however, showed peculiar restriction specificities. Thus, these clones responded to KLH in the presence of (BALB/c × B6E6)F1 APCs but not in the presence of CBF1 APCs. The restriction elements for these clones are not explainable by the conventional knowledge of H-2 genetics since the specificity of class II molecules expressed on (BALB/c × B6E6)F1 and CBF1 APCs should theoretically be the same (i.e., A\(^b\)E\(^a\), A\(^b\)E\(^a\), E\(^b\)E\(^a\), E\(^b\)E\(^a\), A\(^b\)A\(^a\), A\(^b\)A\(^a\), and A\(^b\)A\(^a\)). The frequency of clones that showed such peculiar restriction specificity was ~10% from our limiting dilution cloning.

mAb Blocking Experiments of Clone KTF2.7. Since the only difference between (BALB/c × B6E6)F1 and CBF1 mice is the presence or absence of E\(^a\) transgenes,

| Table I |
|-----------------|-----------------------|-----------------------|
| T cell lines and clones | (BALB/c × B6E6)F1 | CBF1 |
|-------------------|-------------------|------------------|
| KTF1             | 564 ± 89*         | 4,724 ± 1,163     | 1,039 ± 189      | 6,195 ± 933 |
| KTF1.1           | 113 ± 23          | 6,681 ± 1,114     | 169 ± 22         | 7,359 ± 897 |
| KTF2.1           | 304 ± 33          | 9,204 ± 471       | 68 ± 28          | 8,497 ± 122 |
| KTF1.2           | 230 ± 251         | 4,717 ± 2,971     | 150 ± 57         | 582 ± 173  |
| KTF2.7           | 587 ± 35          | 9,349 ± 341       | 573 ± 17         | 897 ± 55   |

10^4 T cell lines or clones were stimulated with 50 µg/ml of KLH in the presence of 10^6 APCs from (BALB/c × B6E6)F1 or CBF1 mice. Cells were cultured for 2 d. 0.5 µCi of [3H]TdR was added 16 h before the termination of culture. Proliferative responses were assayed by the standard scintillation counting. Results are expressed by the mean cpm of the triplicate cultures ± SD.

* [3H]TdR uptake.
it is possible that such Ed transgene products associate with endogenous A\text{d} or A\text{b} gene products to form mixed isotype A\text{d}E\text{d} or A\text{b}E\text{d} molecules that are expressed on (BALB/c × B6Ed\text{d})F1 but not on CBF1 spleen cells. One of the possible explanations for the restriction specificity of clones KTF1.2 and KTF2.7 would be that they use such mixed isotype A\text{d}E\text{d} or A\text{b}E\text{d} molecules as restriction elements. To demonstrate this, we performed mAb blocking experiments using a large panel of chain-specific anti-class II mAbs. As shown in Table II, the proliferative response of clone KTF2.7 was blocked by both anti-A\text{d} and anti-E\text{a} mAbs. None of mAbs having specificities against A\text{a} or E\text{a} could block the clone KTF2.7. Furthermore, anti-I-A mAbs having specificities other than A\text{d} could not block the clone KTF2.7. These mAbs could block the proliferation of appropriate T cell clones at the concentrations used in this experiment (data not shown). This result strongly suggests that clone KTF2.7 uses mixed isotype A\text{d}E\text{d} molecules as restriction elements. Our preliminary experiments that demonstrated the ability of APCs from both A\text{d} and E\text{d} gene-introduced B6 double-transgenic mice to present KLH to clone KTF2.7 (Mineta, T., M. Matsunaga, K. Seki, J. Miyazaki, M. Uno, K. Yamamura, and M. Kimoto, manuscript in preparation) also support this.

### Table II

mAb Blocking of A\text{d}E\text{d}-restricted Clone KTF2.7

| Exp. | mAb (references) | Specificity | Dilution of mAb | cpm |
|------|------------------|-------------|-----------------|-----|
|      |                  |             | 1 x 3 x 9 x 27  |     |
| 1    | 25-9-17 (16,17)   | A\text{d}d  | 215 3,573 4,589 5,299 |
|      | MK-D6 (18,19)     | A\text{d}  | 722 3,827 6,661 8,305 |
|      | 34-5-3 (17,20)    | A\text{d}d  | 653 3,442 5,406 6,695 |
|      | Y17 (21)          | E\text{d}E\text{a} | 8,171 8,119 8,055 7,601 |
|      | 17-3-3 (22)       | E\text{d}  | 8,324 9,438 8,619 8,925 |
|      | 13/4 (23)         | E\text{a}  | 359 2,505 5,996 7,462 |
| 2    | 3JP (17,24)       | A\text{d}d  | 7,151 6,978 7,129 7,225 |
|      | 3F-12 (24)        | A\text{d}  | 7,380 7,483 7,032 7,163 |
|      | 25-5-16 (16)      | A\text{b}  | 7,395 6,106 7,555 7,303 |
|      | 17/227 (6,23)     | A\text{d}d  | 876 2,060 3,642 3,835 |
|      | ISCR-3 (23)       | E\text{a}  | 635 1,031 2,679 3,154 |
| 3    | K24-199 (26,27)   | A\text{d}d  | 5,084 5,152 4,832 5,188 |
|      | 28-16-8 (16,17)   | A\text{d}d  | 530 815 948 1,148 |
|      | 25-9-3 (16)       | A\text{b}  | 5,155 5,125 5,074 5,711 |
|      | 34-1-4 (20)       | E\text{d}  | 5,086 4,998 4,603 5,157 |

\textsuperscript{10}\textsuperscript{4} cells of clone KTF2.7 were stimulated with 50 \mu g/ml of KLH in the presence of APCs from (BALB/c × B6Ed\text{d})F1 mice. 20 \mu l of varying dilutions of mAbs (10-fold concentrated from culture supernatants by 50% ammonium sulfate precipitation) were added at the initiation of culture. Proliferative responses were assayed as described in Table I. The SD of each mean is within 15%. Proliferative responses in the absence and presence of KLH are 587 and 8,810 cpm (Exp. 1), 964 and 7,832 cpm (Exp. 2), and 199 and 5,404 cpm (Exp. 3), respectively.

\textsuperscript{*} References with the original descriptions and the assignment of chain specificities of mAbs.

\textsuperscript{1} Specificities only related to this experiment are shown.

\textsuperscript{5} [\textsuperscript{3}H]TdR uptake.
Discussion

The existence of mixed isotype class II molecules was demonstrated in L cell transfectants (5-8) and in murine and human B lymphoblastoid cell lines (9-11). The possible existence of mixed isotype class II molecules was also reported in several strains of mice (4, 12). Some of these studies suggested that pairing efficiency of isotype-mismatched α and β chains is low compared with isotype-matched α-β pairing (7, 8). This could be the reason why the mixed isotype class II molecule is difficult to detect under the normal situation where the full set of α and β chains exist. Expression of isotype-mismatched α-β pairing seems to occur under the situation where one of the isotype-matched α or β chains does not exist (5-9) or the expression of the isotype-matched α and β chains is unbalanced (10, 11). Transfection experiments of antisense mRNA by Lotteau et al. (11) clearly demonstrated that this is the case. The results in this paper also support this because B6Ea transgenic mice contain 20-40-fold more Ea transgene products per cell than normal mice (13). The frequency of mixed isotype-restricted T cell clones was estimated to be ~10% from our limiting dilution cloning. Also, mixed isotype-restricted clones sometimes showed poor proliferative responses against appropriate stimulus (12, and this paper). These would reflect the small amount of such mixed isotype-restricted molecules expressed on (BALB/c × B6Ea)F1 mice.

The significance of such mixed isotype class II molecules in relation to the expansion of the T cell specificity repertoire, as well as the association of disease susceptibility to HLA haplotypes, has already been pointed out by others (15). It would be possible that certain viral infections could induce unbalanced expression of isotype-matched α and β chains, which in turn result in the formation and expression of a certain allelic combination of isotype-mismatched α-β-paired molecules. This could occur not only on the APCs but also on cells that usually do not express class II molecules. Such molecules might be recognized as "non-self" and would be the target for T cell-mediated autoimmune attack. Alternatively, mixed isotype class II molecules may have high affinity for self antigens and T cells with specificity for self antigens, plus newly expressed class II molecules induce or amplify the organ-specific autoimmunity. The situation would be more exaggerated under the local release of lymphokines such as IFN-γ, IL-4, and TNF from activated T cells.

Summary

Mixed isotype AβEα class II molecule-restricted antigen-reactive T cell clones were obtained from (BALB/c × B6Ea)F1 mice. These T cell clones responded to keyhole limpet hemocyanin in the presence of (BALB/c × B6Ea)F1 but not CBF1 APCs. Both anti-Aβ and anti-Eα mAbs blocked the proliferative responses of these clones. The frequency of such mixed isotype AβEα-restricted T cell clones in (BALB/c × B6Ea)F1 mice was estimated to be ~10% from our limiting dilution cloning. The existence of such mixed isotype class II molecule-restricted T cells would have important implications for the expansion of the T cell repertoire as well as the induction of autoimmunity.

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