T-cell receptor-specific monoclonal antibodies against a V\(_{\beta}11\)-positive mouse T-cell clone

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Abstract. Two monoclonal antibodies specific for the mouse T-cell receptor (Tcr) have been established by immunization with a V\(_{\beta}11\) T-cell clone, clone C6. One is a rat antibody, KT11 (IgG2b, k), specific for the V\(_{\beta}\) chain of C6, V\(_{\beta}11\). This was demonstrated by the fact that the strain distribution pattern of KT11\(^+\) cells was similar to that of V\(_{\gamma}5\), 8, 9, 11, 12, and 13 and that the gene that encodes the molecule detected by KT11 was closely linked to V\(_{\gamma}8\) in (B10 × SJL)F\(_1\) × SJL backcross mice. Furthermore, V\(_{\gamma}\) of C6 has been cloned from a λgt10 cDNA library and was demonstrated to be identical to the V\(_{\gamma}11\) published sequences. All strains of mice that do not express major histocompatibility complex class II E molecules had higher numbers of KT11\(^+\) cells than E\(^+\) strains. The KT11\(^+\) population in A strain mice and its H-2 congenic strains, however, was not affected by the presence or absence of E molecules. The other is a mouse antibody, KTL2 (IgM), specific for the idiotope of the Tcr expressed on the clone C6. Both antibodies were mitogenic and induced cytotoxicity. Expression of epitopes detected by KT11 or KTL2 was down-modulated by a T3e-specific antibody 145-2C11.

Introduction

Antibodies specific for the variable region of the β chain (V\(_{\beta}\)) of the mouse T-cell receptor (Tcr) have attracted substantial attention since Kappler and Marrack and their colleagues showed that V\(_{\beta}17a\) is expressed predominantly on T cells specific for major histocompatibility complex (MHC) class II E molecules and that the majority of V\(_{\beta}17a\) cells are eliminated from the T-cell repertoire of mice expressing E molecules (Kappler et al. 1987a, b). This has given significant impetus to the clonal deletion hypothesis in the induction of T-cell tolerance. Subsequently, similar evidence has been reported that V\(_{\gamma}8.1\) and V\(_{\gamma}6\) are predominantly expressed on T cells specific for Mls\(^a\), and that these cells are deleted from Mls\(^a\)-expressing strains of mice (Kappler et al. 1988, MacDonald et al. 1988).

In this paper the establishment and initial characterization of a V\(_{\beta}11\)-specific antibody as well as an antibody specific for an idiotope of a Tcr are reported.

Materials and methods

Animals. Mice and Sprague-Dawley outbred rats described in this paper were obtained from the Clinical Research Centre, the National Institute for Medical Research and Olac, Bicester, England.

Medium. RPMI 1640 was supplemented with penicillin/streptomycin, 10 mM Hepes, and 5 × 10\(^{-5}\) M 2-mercaptoethanol. Ten percent fetal calf serum (FCS) and 10% culture supernatant of rat spleen cells stimulated by Con A prepared as described (Tomonari 1983) were added to medium for maintenance of T-cell clones. Fifteen percent FCS was added to medium for proliferation assays.

T-cell clones. T-cell clones used in this paper are shown in Table 3. Clones 2-1, 10-2, 4, A1, A3, B1, B9, C6, F5, T5, and T16 have been described in previous papers (Tomonari 1983, 1985a, 1985b, 1987, 1988b).

Antibodies. A hybridoma secreting an antibody KT11 (IgG2b, k) and a hybridoma secreting an antibody KT15 (IgG2a, λ) specific for Ly-2 were established from a fusion between NSO myeloma cells and spleen cells from a Sprague-Dawley rat hyperimmunized with a T-cell clone, clone C6. A hybridoma secreting an antibody KTL2 (IgM) was established from a fusion between a NSO myeloma cell and spleen cells from a C3H/He mouse hyperimmunized with the clone C6. A rat antibody KT3 (IgG2a, λ) specific for the Tcr/T3 complex and a rat antibody KT12 (IgG2b, k) specific for H-2\(^\kappa\) have been reported in a previous paper (Tomonari 1988b). A hybridoma secreting a V\(_{\gamma}1.8.2\)-specific antibody KJ16 (Haskins et al. 1984) was obtained from Dr. P. Marrack, Howard Hughes Medical Institute, Denver, Colorado. A hybridoma secreting a T3e-specific antibody 145-2C11 (Leo et al. 1987) was obtained from Dr. J. Bluestone. A hybridoma secreting an antibody MAR18.5 specific for rat Ig kappa chain was obtained from the American Tissue Typing Collection (Rockville, Maryland). Culture super-
natants from these hybridomas were used as antibodies. These hybridomas/antibodies are available upon request.

**Proliferation assay.** Ten thousand clone C6 cells and various concentrations of KT11 or KTL2 were incubated in the presence or absence of an Ly-2-specific antibody KT15 in 250 μl medium supplemented with 10 ng/ml phorbol myristate acetate (PMA) for 72 h; 3H-thymidine (18.5 kBq/well) was added for the final 12 h.

**Cytotoxicity assay.** Ten thousand 51Cr-labeled hybridoma cells were incubated with various numbers of cloned T cells in 200 μl of medium for 4 h, and 100 μl of supernatant was assayed for 51Cr release. Ten thousand 51Cr-labeled P815 mastocytoma cells in 100 μl of medium and various numbers of cloned T cells in 100 μl of medium were incubated in the presence or absence of 10 μl of antibody (KT11, KTL2, KT3, or KT15) for 4 h, and 100 μl of supernatant was assayed for 51Cr release.

**Analysis with a fluorescence-activated cell sorter (FACS).** Con A blast cells were prepared in the following way: 1.5 x 10⁶ spleen or lymph node cells were cultured in 10 ml medium supplemented with 50 units/ml recombinant IL 2, 1 μg/ml Con A, and 10% FCS for 5 days. The Con A blast cells and freshly prepared lymph node cells were stained with fluorescein isothiocyanate (FITC)-coupled 145-2C11 antibody or stained with FITC-coupled MAR18.5 antibody after treatment with KT11 antibody as described previously (Tomonari 1985a) and analyzed with a FACS (FACStar). T-cell clones were directly stained with KT11/FITC or KTL2/FITC and analyzed with the FACS. Down-modulation of the Tcr/T3 complex by 145-2C11 antibody was carried out as described (Tomonari 1985a). Briefly, after incubation of clone C6 cells

| Table 1. Mitogenic activity of antibodies KT11 and KTL2* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Experiment     | Supplement      | Concentrations of KT11 in the culture |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1.              | None            | 1/16 1/256 1/4096 None |
| Anti-Ly-2       | 1.859 ± 531     | 729 ± 333 155 ± 49 50 ± 14 |
| 2.              | None            | 1/16 1/256 1/4096 None |
| Anti-Ly-2       | 1.222 ± 78      | 1116 ± 676 211 ± 6 115 ± 75 |

Concentrations of KTL2 in the culture

| 1/16 1/256 1/4096 None |
|-----------------------|-------------------|
| 3.                     | 6.597 ± 1676 8.257 ± 652 12.530 ± 823 7.971 ± 1262 94 ± 17 |
| 4.                     | 21.517 ± 2.142 20.756 ± 2.069 34.354 ± 3.124 1.24 ± 0.7 124 ± 69 |
| 5.                     | 15.971 ± 2.875 25.550 ± 2.454 28.445 ± 100 ND 64 ± 11 |

* Clone C6 cells were cultured with various concentrations of antibodies, KT11 or KTL2, in the presence of PMA. An Ly-2 specific antibody KT15 at a concentration 1/1000 was added to some of the cultures stimulated with KT11. Data were expressed as mean cpm of triplicates ± standard deviation

| Table 2. Induction of cytotoxicity by antibodies KT11 and KTL2* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Experiment     | Target          | Effector        | Effector to target cell ratios |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1.              | KT11 hybridoma  | AK1 52.1 ± 4.5 41.4 ± 4.2 17.2 ± 2.8 8.3 ± 1.0 |
|                 | AK5 1.0 ± 1.6 0.0 ± 0.7 0.1 ± 0.8 0.5 ± 0.6 |
|                 | AK6 53.8 ± 1.5 46.6 ± 2.3 29.7 ± 1.0 10.5 ± 1.0 |
| KT3- hybridoma  | AK1 55.6 ± 1.2 50.8 ± 1.6 38.9 ± 1.1 20.0 ± 1.5 |
|                 | AK5 43.3 ± 2.5 36.2 ± 1.8 28.2 ± 3.0 12.4 ± 2.1 |
|                 | AK6 59.4 ± 3.0 57.4 ± 4.0 43.2 ± 2.7 23.6 ± 3.3 |
|                 | C6 40.5 ± 3.1 37.4 ± 0.6 26.1 ± 0.9 11.8 ± 0.7 |
| 2.              | P815            | C6+KT11 69.7 ± 2.3 52.7 ± 4.6 34.7 ± 1.7 14.3 ± 0.7 |
|                 | C6+KTL2 17.8 ± 0.8 14.7 ± 0.7 8.7 ± 1.4 3.1 ± 0.5 |
|                 | C6+KT3 58.3 ± 2.8 44.0 ± 3.3 27.9 ± 3.5 12.9 ± 0.7 |
|                 | C6+KT15 4.0 ± 0.7 2.0 ± 0.2 0.6 ± 0.4 0.3 ± 0.8 |
|                 | C6+medium 2.7 ± 0.7 0.0 ± 0.2 0.1 ± 0.6 0.3 ± 1.1 |

* 51Cr-labeled target cells were incubated with cloned T cells for 4 h. 10 μl/well of antibodies (KT11, KTL2, KT3, KT15, or medium) were supplemented in experiment 2, but not experiment 1. A Tcr/T3-specific antibody KT3 and an Ly-2-specific antibody KT15 were used as controls

* Percent cytotoxicity ± standard deviation