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A novel monoclonal reagent recognizing native and denatured $V_{\beta 5.3}$-related chains of human T cell receptor

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Abstract

Monoclonal antibodies to specific families of TCR variable domains serve as highly useful immunochemical tools for basic research in T-cell biology and diagnosis of autoimmune diseases. Monoclonal antibody MEM-262 characterized in this communication recognizes β chains of the TCR expressed by HPB-ALL cell line (carrying $V_{\beta 5.3}$) and a small subset of peripheral blood T cells. This subset is larger than that recognized by a previously described $V_{\beta 5.3}$-specific mAb. MEM-262 potently stimulates selective expansion of the T-cell subset, efficiently immunoisolates native TCR complexes as well as free β chains and uniquely recognizes denatured TCRβ chains under the conditions of Western blotting.

Keywords: Monoclonal antibody; T-cell receptor; T-cell receptor β chain; Variable domain; HPB-ALL; $V_{\beta 5.3}$

The T cell receptor for antigen (TCR) is a disulfide-linked heterodimer composed of two type I integral membrane glycoproteins: α and β (human major T-cell population) or γ and δ. The α/β TCR is expressed by the majority of T cells in human and binds antigens presented by molecules of major histocompatibility complex (MHC) [1]. The extracellular part of α and β TCR chains consists of two immunoglobulin-like domains: a membrane-distal V- (variable) domain and a membrane proximal C- (constant) domain. Genes encoding variable regions are assembled from V and J (in case of V_α) or V, D and J (in case of V_β) gene segments through a somatic recombination process termed V(D)J recombination [2]. Several other molecules, together designated the CD3 complex, are closely associated with TCR heterodimer; intracellular parts of these transmembrane proteins (γ, δ, ε, ζ/η) contain immunoreceptor tyrosine-based activation motifs and are responsible for signal transduction from TCR inside the cell [1].

It has been reported that certain human as well as animal autoimmune diseases are associated with specific V_β-restricted repertoires [3–6]; in these cases monoclonal antibodies are highly useful tools for distinguishing the various categories of TCR and the particular constant and variable domains. Antibodies recognizing selectively specific V_β are also useful for studies on T cell ontogenesis [7–9]. In this report, we describe unique features of a novel monoclonal antibody MEM-262 directed against TCR possessing $V_{\beta 5.3}$-related chains.

Human cell lines used in this work were obtained from our Institute's cell line collection. T lymphocytes were purified from peripheral blood mononuclear cells on a nylon wool column [10]. For long-term activation, the purified T cells (5 million in 5 ml RPMI with 10% fetal calf serum and 500 IU IL-2) were plated into 5 cm plastic culture dishes precoated with purified mAb MEM-262 or control anti-CD3 mAb MEM-57. Cells were then cultured for 3 days, harvested, washed and further cultured 14 days in clean (non-coated) culture dishes in the presence of 100 IU/ml IL-2. Most reagents were purchased from Sigma (St. Louis, MO), anti-Ig-horseradish peroxidase conjugate was from Bio-Rad (Hercules, CA), enhanced chemiluminescence Western blotting kit, CNBr-activated Sepharose 4B from Amersham Biosciences (Little Chalfont, UK) and laurylmal-

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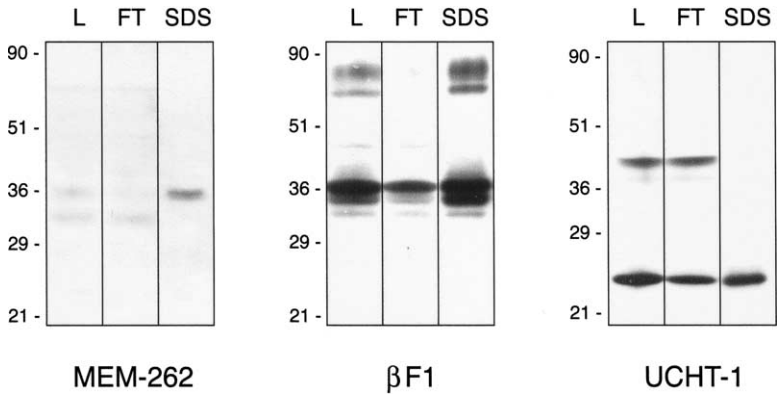

Fig. 1. Immunoaffinity chromatography on the MEM-262 immunosorbent. HPB-ALL cells were solubilized in the presence of 1% laurylmaltoside and subjected to immunoaffinity chromatography on a minicolumn made of immobilized mAb MEM-262; bound proteins were eluted with 2 × concentrated SDS-PAGE non-reducing sample buffer. Cell lysate (L), flow-through fraction (FT) and eluted fractions (SDS) were analyzed by non-reducing SDS-PAGE followed by Western blotting using the indicated mAbs (βF1 to β chain of TCR, UCHT-1 to CD3ε). Positions of molecular weight standards (in kDa) are indicated at the left margins.

toside (*n*-dodecyl-β-D-maltoside) from Calbiochem (San Diego, CA). Mouse hybridoma secreting mAb MEM-262 (IgG2a) was prepared using standard techniques from splenocytes of mice immunized with human T-cell line HPB-ALL; mAbs MEM-43/5 (IgG2b; anti-CD59), MEM-57 (IgG2a; anti-CD3ε), P-Tyr-01 (IgG1; anti-phosphotyrosine) and P-Tyr-02 (IgG2b; anti-phosphotyrosine) were prepared and characterized in our laboratory as well. MAb βF1 (IgG1; anti-TCRβ)? was kindly provided by Dr M. Brenner (Harvard University, Boston, MA) and mAb UCHT-1 (IgG1; anti-CD3ε) by Dr P. Beverley (ICRF, London, UK). MAb 3D11 (IgG1; anti-TCR$V_{\beta 5.3}$) was purchased from Beckman Coulter (Fullerton, CA). Phycoerythrin (PE) or fluorescein labeled monoclonal antibodies to CD3, CD4, CD8, CD19 were from Diaclone (Besancon, France). Purified mAb MEM-262 was fluorescently labeled by FITC using a standard procedure. For flow cytometry, cells were stained with fluorescently labeled or unlabeled mAbs (20 μg/ml) for 30 min on ice, washed and, if appropriate, incubated with fluorescein-labeled goat $F(ab')_2$ anti-mouse Ig (Jackson Immunoresearch, West Grove, PA) and analyzed on a FACSort flow cytometer (Becton Dickinson, Mountain View, CA) in a standard setup. Preparation of cell lysates, SDS-PAGE and Western blotting (employing luminographic detection) were all performed essentially as described in detail elsewhere [11]. Protein A-purified mAb MEM-262 was covalently bound to CNBr-activated Sepharose according to manufacturer's instructions. Immunoaffinity chromatography was performed at 4 °C on minicolumns (50 μl packed volume) of the immunosorbents washed thoroughly with the lysis buffer containing 1% detergent laurylmaltoside, bound proteins were gradually eluted with four column volumes of alkaline buffer (0.1 M glycine NaOH, pH 11.5, containing 0.1% NP-40) and four column volumes of 2 × concentrated SDS-PAGE non-reducing sample buffer; in some cases the alkaline elution was omitted. Fractions eluted by alkaline buffer were neutralized, all samples were mixed 1:1 with 2 × concentrated SDS-PAGE non-reducing sample buffer, boiled and analyzed by SDS-PAGE followed by Western blotting. In the case of reduced samples, dithiothreitol was added to final concentration 0.5% before boiling.

Spleen cells of mice immunized with human T cell line HPB-ALL were used for hybridoma construction. One of the mAbs, MEM-262, strongly reacted with the HPB-ALL cells but not with any other cell line tested (Jurkat, KG1a, Raji). Moreover, it recognized a small subpopulation of peripheral blood T cells (less than 1%), both CD4- and CD8-positive (data not shown). This pattern of expression suggested that the mAb might be directed to a variable region of α or β chains of the TCR expressed by HPB-ALL cells and shared also by a subset of peripheral blood T cells. To prove this possibility, MEM-262-based immunosorbent was used for immunoprecipitation of the respective antigen from detergent lysate of HPB-ALL cells under the conditions largely preserving integrity of the TCR/CD3 complex. The material eluted from the immunosorbent by SDS contained a protein of 36 kDa that was specifically immunostained by the MEM-262 mAb under the conditions of Western blotting (non-reduced sample only) and co-migrated exactly with the zone immunostained by a standard mAb directed to TCRβ chains (Fig. 1). Furthermore, as expected, CD3ε chain was coprecipitated on the MEM-262 immunosorbent (Fig. 1) and could be eluted already by high pH. It can be seen from the immunostaining with the pan-TCRβ-chain specific antibody (Fig. 1, middle panel) that the MEM-262 immunosorbent removed from the HPB-ALL cell lysate nearly quantitatively also a zone migrating at approxi-

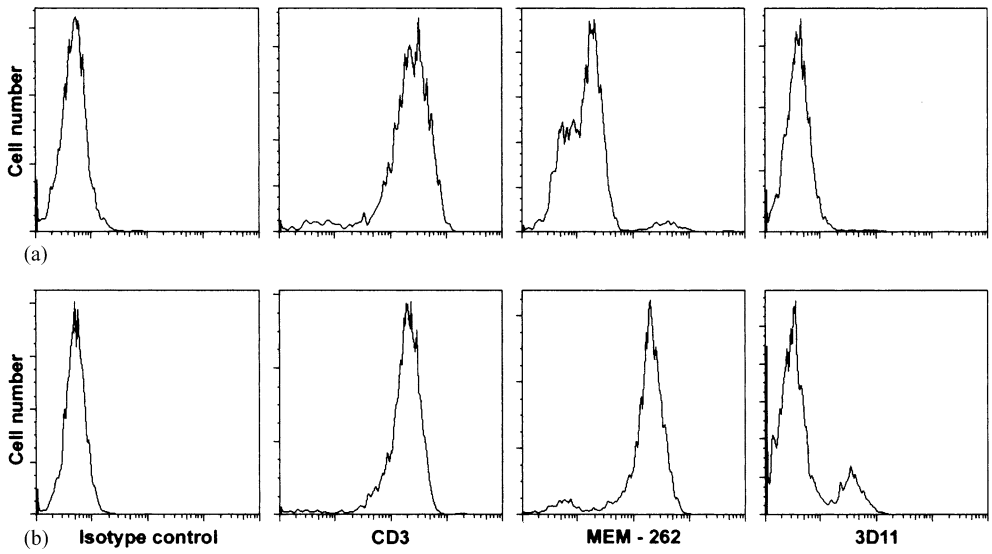

Fig. 2. Expansion of MEM-262-positive T lymphocytes in vitro. Peripheral blood T cells were stimulated for 3 days by immobilized MEM-57 (part a; anti-CD3 control) or MEM-262 (part b) mAbs and IL-2 and then 14 days in the presence of IL-2 only, and immunostained with the indicated mAbs.

mately 75 kDa (obviously the covalent heterodimer of the TCR α and β chains).

These data strongly suggest that MEM-262 recognizes the TCRβ subunit of 36 kDa expressed in the HPB-ALL cells, either free or within the TCR/CD3 complex. HPB-ALL cells are known to carry the $V_{\beta5.3}$ chains, and therefore, MEM-262 must be more or less specific for this V_β species. In this respect, it may be similar to previously described mAbs [12,13].

The epitope recognized is unusually resistant to denaturation by SDS and thus can be detected by Western blotting. It is remarkable that under the conditions of Western blotting (non-reduced samples) the mAb recognizes apparently only the free β chain although the major species detected under these conditions should be the covalent (disulfide-bound) αβ dimer (which is detected as the 75–80 kDa zone by the pan-TCRβ-specific mAb). It is evident that HPB-ALL cells contain an excess of free (presumably cytoplasmic) β chain which is recognized better (at least under the conditions of Western blotting) by the mAb than that present in the αβ dimers.

Next we compared MEM-262 with another previously described mAb of similar specificity, 3D11 [13]. 3D11 reacted with HPB cells; FITC-labeled MEM-262 did not compete (cross-block) with 3D11; double staining of peripheral blood T cells with both these mAbs revealed that they recognize similar, partially overlapping subsets of T cells, MEM-262 being reactive with a larger population including essentially all 3D11-reactive cells (data not shown). When peripheral blood T cells were stimulated by immobilized MEM-262, the subpopulation reactive with the mAb selectively proliferated and after 17 days most of the cells were MEM-262-positive (Fig. 2b); interestingly, nearly 90% of them were CD8-positive, while only a minority were CD4-positive (not shown). Only a fraction (ca. 20%) of these expanded cells were recognized by the 3D11 mAb (Fig. 2b), demonstrating again that these two mAbs are not identical in their specificity. No selective expansion of the MEM-262-positive cells was observed if immobilized anti-CD3 mAb was used for stimulation (Fig. 2a). Thus, the mAb MEM-262 can be used for selective in vitro expansion of the T cells carrying the $V_{\beta5.3}$-related TCR.

It can be concluded that mAb MEM-262 is directed to the TCR $V_{\beta5.3}$ chains expressed on HPB-ALL cells and a small subset of peripheral blood T cells. This subset is larger than that recognized by a previously described $V_{\beta5.3}$-specific mAb. The epitope is relatively resistant to denaturation, so the mAb can be used not only for immunoisolation of native TCR complexes and free β chains but also for specific detection of non-reduced $V_{\beta5.3}$-related TCR chains under the conditions of Western blotting. Furthermore, the mAb is mitogenic and can be used for specific expansion of the T cells carrying the $V_{\beta5.3}$-related TCR chains. Thus, the mAb MEM-262 may serve as a novel useful V_β-specific tool.

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