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The cytotoxic analysis of T cell receptor V$\delta 1^+$ T cell lines derived from the synovial fluid of rheumatoid arthritis patients

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SUMMARY

We established six human T cell lines derived from rheumatoid arthritis synovial fluid (RASF). Phenotypically, T cell receptor (TCR) $\gamma\delta$ T cells occupied the majority of these lines and most of them expressed the TCR V$\delta 1$ molecule. In contrast, V$\delta 2^+$ T cells, the majority population of peripheral blood $\gamma\delta$ T cells, were rarely detected in these lines. To study the immunobiological roles of RASF V$\delta 1^+$ T cells in RA development, their cytotoxic profile was studied. The results showed that these T cells selectively lysed Daudi, but not K562 cells. The cytotoxic response was MHC-unrestricted, and was inhibited by anti-CD3 MoAb. Moreover, the cold target inhibition assay showed that the cytotoxicity was competitively inhibited by autologous and allogeneic primarily cultured RA synovial cells as well as synovial sarcoma and chondrosarcoma lines. However, PBL did not inhibit this cytotoxicity. These data suggest that V$\delta 1^+$ T cells in RASF may recognize the antigen which is commonly expressed on the surface of Daudi and the cells derived from RA synovium. We can assume that the cytotoxic V$\delta 1^+$ T cells are selectively expanded in RASF, playing a significant role for the pathogenesis of certain RA cases.

Keywords $\gamma\delta$ T cells cytotoxicity rheumatoid arthritis synovial fluid

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease which mainly affects the joints. Although the pathogenesis of this disease is assumed to be of an autoimmune nature, its etiology is still unknown. Recently, several investigators have described $\gamma\delta$ T cell clones derived from RA synovial fluid (RASF), some of which reacted with mycobacterium extract or the heat shock protein (hsp) [1–3]. These investigations suggested that hsp-reactive $\gamma\delta$ T cells may have been expanded in the RA synovial tissue.

Meanwhile, it has been reported that $\gamma\delta$ T cells in RASF might be somewhat unusual in their population. Although V$\delta 1^+$ T cells are usually a minor population among peripheral $\gamma\delta$ T cells, immunohistochemical and FACS analyses showed that V$\delta 1^+$ T cells might be extraordinarily enriched in RA synovial membrane and synovial fluid in comparison with peripheral blood lymphocytes (PBL) [4–8]. It was also shown that V$\delta 1^+$ T cells were predominant in coeliac jejunum [9] and in the blood of AIDS patients [10], so it is likely that this enhancement may reflect an important immunobiological role of V$\delta 1^+$ T cells in the chronic inflammatory process. Furthermore, a recent report suggested that V$\delta 1^+$ T cells in RASF were stimulated *in vivo*, and were clonally expanded in the synovial tissue [8]. However, the reproducibility and immunobiological significance of this V$\delta 1^+$ T cell predominance in RA joint inflammation are entirely unknown.

In our present investigation, we successfully cultured *in vitro* T cell lines derived from RASF. We found that in all six RA cases the V$\delta 1^+$ T cells were predominantly expanded *in vitro*. In contrast, V$\delta 2^+$ T cells, the most predominant population among human peripheral $\gamma\delta$ T cells, were not induced. We considered that these V$\delta 1^+$ T cells in RASF could play a significant role in the destruction of RA joints, since these particular T cells may be directly cytotoxic to the cells which compose the joint structure.

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PATIENTS AND METHODS

Patients

Synovial fluid was obtained from six patients who met the American Rheumatism Association 1987 revised criteria for RA [11]. Peripheral blood was also obtained from two of these patients. All of them were female, 37–61 years old, and the duration of disease was 3–25 years after the first clinical diagnosis was made. The disease was still active and was not burned out when the cells were obtained.

Isolation and establishment of RASF T cell lines

To establish T cell lines from RASF, 5–8 ml of RASF were

centrifuged at 200 *g* at room temperature for 5 min. The pellets were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 292 μg/ml of L-glutamine and 200 U/ml of recombinant IL-2 (Shionogi Pharmaceutical Co., Tokyo, Japan). Subsequently, they were cultured for 10 days, then RASF T cell lines were established. In this procedure, we did not use a gradient centrifugation such as Ficoll–Conray. In a bulky culture, T cells, which were already primed *in vivo*, seemed to be in good condition, since various cells such as synovial cells and some joint cell debris were included. In the cytotoxic assay TCR Vδ1$^+$ T cells were enriched by depleting CD4$^+$ and CD8$^+$ T cells by using microCELLector (AIS, Tokyo, Japan) after culturing for 10 days with the medium described above.

MoAbs and flow cytometry

We used the following MoAbs: anti-CD3 (clone 38.1) was obtained from the American Type Culture Collection (ATCC). Anti-CD4 and anti-CD8 were purchased from Ortho Diagnostic Systems Co. (Tokyo, Japan). Anti-MHC class II (TC8B1) was developed in our own laboratory [12]. Anti-CD16 (MG38) and anti-CD56 (NKH-1) were purchased from Funakoshi Co. (Tokyo, Japan). Anti-TCR $\alpha\beta$ (WT31) and anti-TCR$\gamma\delta$ (TCRδ1) were obtained from Ortho Diagnostic Systems and T Cell Sciences (Cambridge, MA) respectively. In addition, MoAbs reacting specifically for TCR$\gamma\delta$ V region gene products were also used; TiγA (kindly provided by Dr T. Hercend, Villejuif, France), A13 and BB3 (kindly provided by Dr L. Moretta, Genova, Italy) recognize Vγ9, Vδ1 and Vδ2 proteins, respectively. Cells were washed once with ice-cold PBS containing 0·05% NaN_3, and approximately 2×10^5 cells in 0·5 ml of PBS/NaN_3 were incubated with a saturated amount of MoAb for 40 min at 4°C. Cells were washed with PBS/NaN_3 and incubated with FITC-conjugated goat anti-mouse IgG/IgM for 40 min at 4°C. After washing and fixing cells with 1% formaldehyde in PBS, samples were run on a FACStar (Becton Dickinson, Mountain View, CA).

Cytotoxicity assays of Vδ1$^+$ T cell lines and blocking experiments by MoAbs

The procedure followed closely that of our previous papers [13,14]. Briefly, target cells were washed and labelled with ^{51}Cr and incubated for 1·5 h at 37°C. Labelled cells (1×10^4) were washed and incubated in 96-well round-bottomed microtitre plates (Costar no. 3799) and cocultured with effector cells in various E/T ratios for 5 h at 37°C. Daudi and Raji B cell lines as

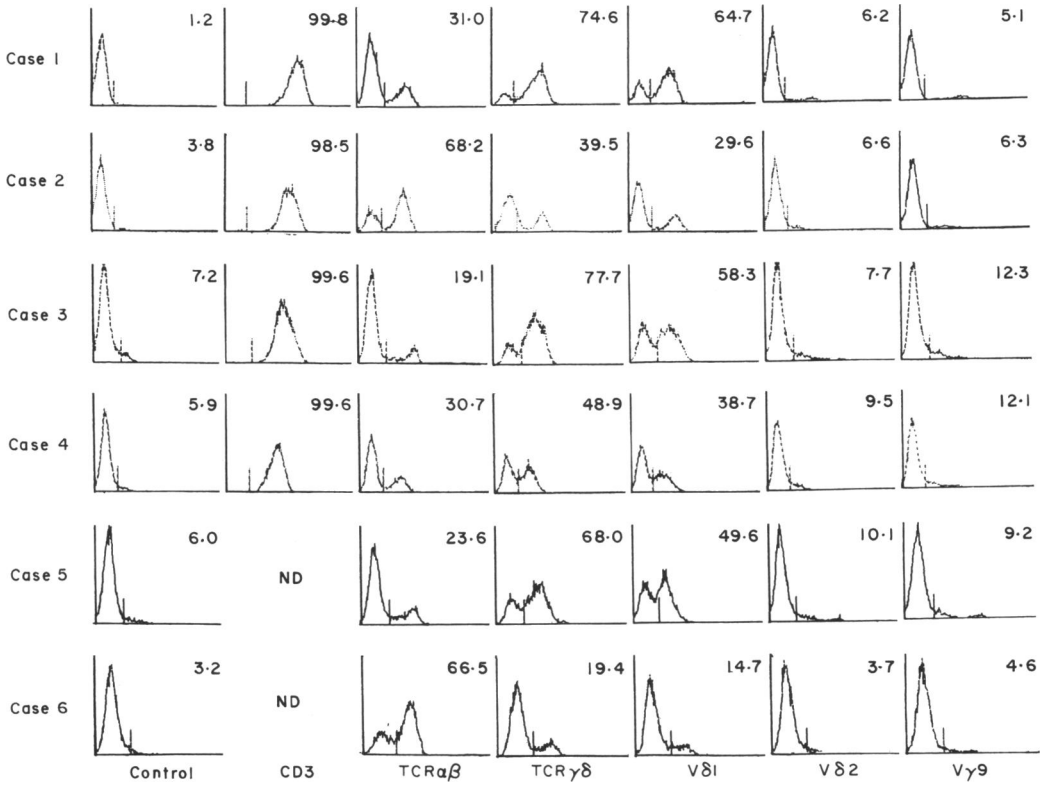

Fig. 1. The FACS analysis of rheumatoid arthritis synovial fluid (RASF) cells cultured primarily *in vitro* for 10 days after obtaining synovial fluid from six patients in the presence of 200 U/ml IL-2. Each number indicates the actual number of per cent positive cells.

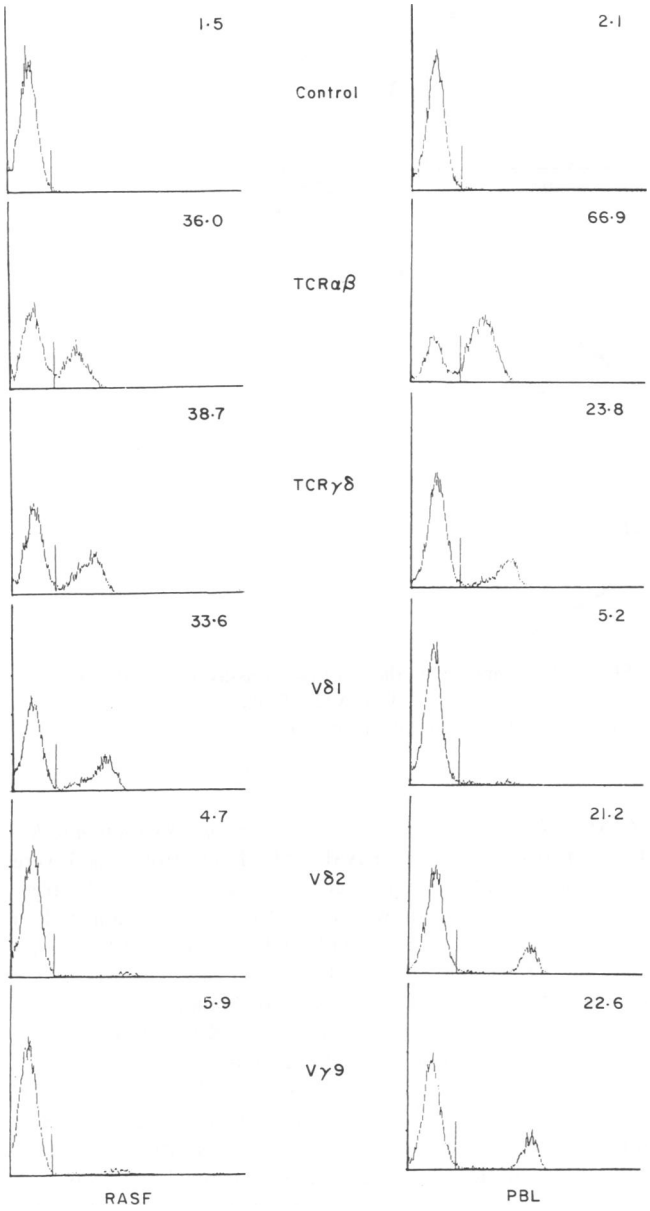

Fig. 2. Comparison of the FACS profiles between rheumatoid arthritis synovial fluid (RASF) cells and peripheral blood lymphocytes (PBL) of case 1. Both cells were cultured primarily for 10 days in the presence of 200 U/ml IL-2. Each number indicates the actual number of per cent positive cells.

well as Molt-4F and K562 leukaemia lines were used as target cells. SW928 synovial sarcoma cell line was also used. The per cent cytotoxicity was calculated as (experimental release−spontaneous release)/(maximum release−spontaneous release)×100. The assays were performed in triplicate in these experiments. Blocking experiments of the cytotoxicity were performed as previously described using anti-CD3 (38.1) and anti-MHC class II (TC-8B1) MoAbs.

Cold target inhibition assays

^{51}Cr-unlabelled competitor target cells were serially diluted to achieve various final hot to cold ratios, and plated in round-bottomed microwells. Then the ^{51}Cr-labelled target cells and effector cells were added. As the cold target cells, we used autologous primarily cultured RA synovial cells. In some experiments, allogeneic PBL and allogeneic primarily cultured synovial tissue cells, which were obtained at the time of a total hip replacement operation, were used. The synovial tissue was minced and treated with 20 μg/ml of collagenase (Sigma Chemical Co., St Louis, MO) for 1 h and cultured for 24 h in a 5% CO_2 incubator. The adherent cells were used as the synovial cells. Usually these primarily cultured cells were cultured for 2–3 days, and were used in the assays. We also employed synovial sarcoma cell line (SW928) and chondrosarcoma cell line (SW1353). These two cell lines were obtained from ATCC.

RESULTS

Cell population of RASF

We first analysed the cell surface phenotypes of RASF cells. The results of six RA cases showed that a relatively high population of γδ T cells was detected in these cells (Fig. 1). It was interesting that almost every γδ T cell expressed Vδ1 but rarely Vδ2 or Vγ9. It is known that Vγ9⁺/Vδ2⁺ T cells are the most predominant T cell population in human peripheral γδ T cells [15]. However, in RASF cells, they seemed to be a rather minor population. We do not know if this was true for non-RASF such as osteoarthritis, since these clinical cases were difficult for obtaining synovial fluid to use in the experiments. On the other hand, when PBL (case 1) were cultured in the same condition, almost every γδ T cell expressed Vδ2 and Vγ9 (Fig. 2). This was true for case 6 (data not shown). Therefore, these data may support the notion that Vδ1⁺ T cells in RASF may be primed and expanded *in vivo* in the synovial fluid and/or synovial membrane itself.

Cytotoxic profile of Vδ1⁺ T cells

To determine the immunobiological significance of Vδ1⁺ T cells which were presumed to be the most predominant in RASF cells, cytotoxicity assays were performed against various target cells. The RASF cells from case 1 were enriched to Vδ1⁺ T cells by depleting CD4⁺CD8⁺ T cells. Figure 3 shows a FACS profile of these enriched Vδ1⁺ T cell lines, demonstrating that more than 90% of them were Vδ1⁺ T cells. We then used Vδ1⁺ T cells as effector cells in the cytotoxicity assay. The data showed that these T cell lines efficiently lysed Daudi cells (Fig. 4a, b) and a synovial sarcoma line SW928 (Fig. 4b). The cytotoxic activity against Raji and Molt-4F was low, while K562 was not lysed by Vδ1⁺ T cells. These TCR Vδ1⁺ T cells were not cytotoxic against a human fibroblast line HEPM (data not shown). Furthermore, anti-CD3 MoAb partially blocked the cytotoxicity against Daudi cells, suggesting that Vδ1⁺ T cells may recognize a certain antigen expressed on the surface of Daudi cells, probably by using this Vδ1⁺ TCR molecule (Fig. 5a). In addition, to study whether this recognition was restricted by MHC molecules, we performed the blocking experiment using MoAb of anti-MHC molecules. Because Daudi cells do not express MHC class I, but express MHC class II molecule, the blocking activity of anti-MHC class II MoAb was assessed. As shown in Fig. 5b, this MoAb was not inhibitory to the cytotoxicity of Vδ1⁺ T cells against Daudi cells. It was therefore considered that this recognition was not restricted by MHC molecules.

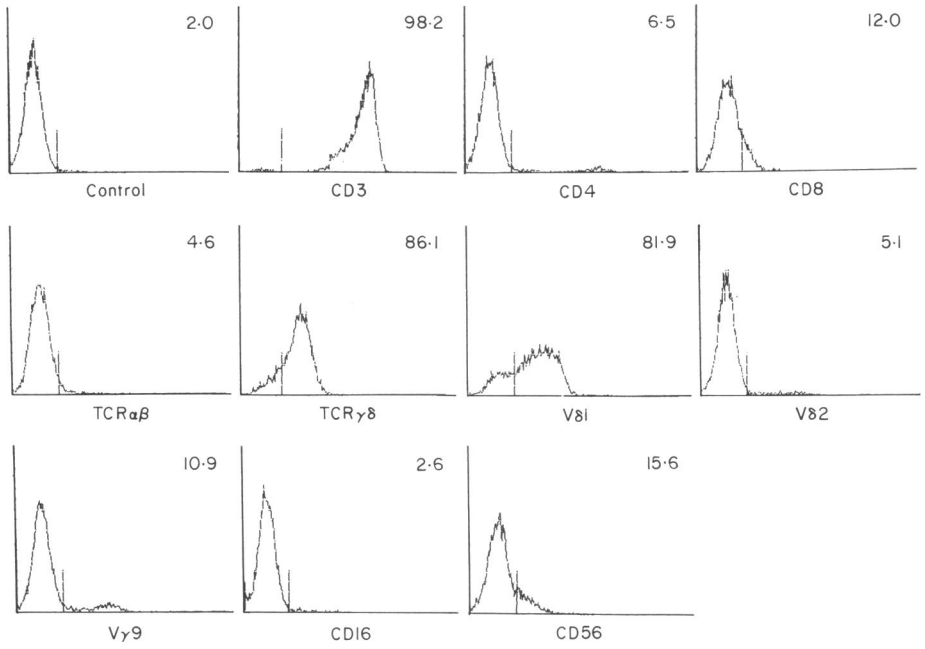

Fig. 3. FACS profile of case 1 rheumatoid arthritis synovial fluid (RASF) cell line employed in the cytotoxicity assay. Case 1 RASF cells in Fig. 2 were continuously cultured for 10 days in the presence of 200 U/ml IL-2. Then, TCR $V\delta1^+$ T cells were enriched by depleting $CD4^+$ and $CD8^+$ T cells, and the cells were used in FACS analysis and the cytotoxicity assay. Each number indicates the actual number of per cent positive cells.

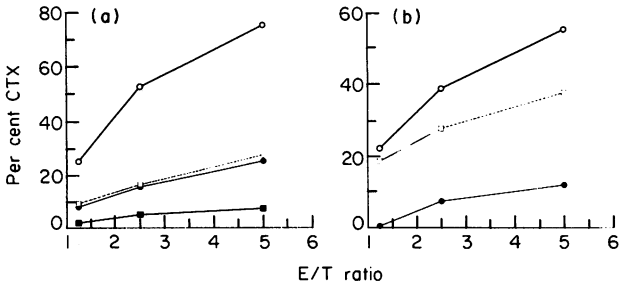

Fig 4. The cytotoxicity (CTX) at different effector to target (E/T) ratios of rheumatoid arthritis synovial fluid (RASF) TCR $V\delta1^+$ T cell line derived from case 1 against ^{51}Cr-labelled (a) Daudi (○), Raji (●), Molt-4F (□) and K562 (■) target cells, and (b) Daudi (○), Raji (●) and SW928 (□).

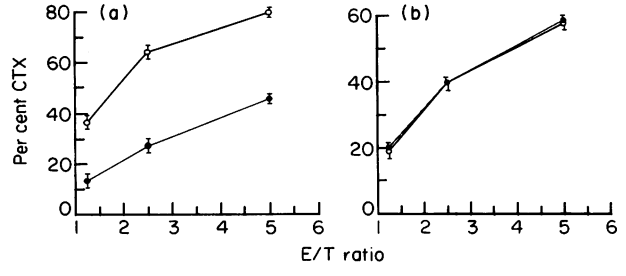

Fig. 5. The blocking experiments by using (a) anti-CD3 MoAb (clone 38.1) (●) and (b) anti-MHC class II MoAb (TC8B1) (●) on the cytotoxicity (CTX) of rheumatoid arthritis synovial fluid (RASF) TCR $V\delta1^+$ T cell line against ^{51}Cr-labelled Daudi cells. Bars represent ± s.e.m. ○, No MoAb.

RASF $V\delta1^+$ T cells may selectively affect joint destruction in RA

In order to study whether RASF $V\delta1^+$ T cells from case 1 were cytotoxic to RA synovial cells, we performed cold target inhibition assays. As shown in Fig. 6a, cold (^{51}Cr-unlabelled) target cells, such as primarily cultured autologous RA synovial cells and Daudi cells, inhibited competitively the $V\delta1^+$ T cell cytotoxicity against hot (^{51}Cr-labelled) Daudi cells, but other cold cell lines such as Raji, Molt-4F and K562 did not. These inhibitions were shown to be dose-dependent (Fig. 6b). Furthermore, it was interesting that cold allogeneic RA synovial cells as well as cold chondrosarcoma SW1353 and synovial sarcoma SW928 lines partially inhibited this cytotoxicity (Fig. 6c). The inhibition by allogeneic RA synovial cells was also dose-dependent (Fig. 6d). As shown in Fig. 7, allogeneic RA synovial cells reproducibly inhibited the cytotoxicity. In contrast, the irradiated PBL derived from the same allogeneic RA patient and from a healthy volunteer did not inhibit the cytotoxicity.

DISCUSSION

In humans, TCR $\gamma\delta$ T cells are present as a minor population in peripheral T lymphocytes. In the peripheral blood, they are usually less than 5%. The TCR $\gamma\delta$ genes could accommodate structurally to very diverse set of antigens such as the TCR $\alpha\beta$ genes. However, it is known that most of these $\gamma\delta$ T cells coexpress heterodimer TCR proteins, comprising $V\gamma9/V\delta2$ molecules, and only a minor subset express $V\delta1$. Although their biological functions are unclear, $\gamma\delta$ T cells have been shown to react with mycobacterial antigens [16–20], including 65-kD heat shock protein (hsp65) [20–22]. Holoshitz *et al.* demonstrated that $\gamma\delta$ T cell clones reacting with the mycobacterial hsp65 were isolated from RASF. They also showed that

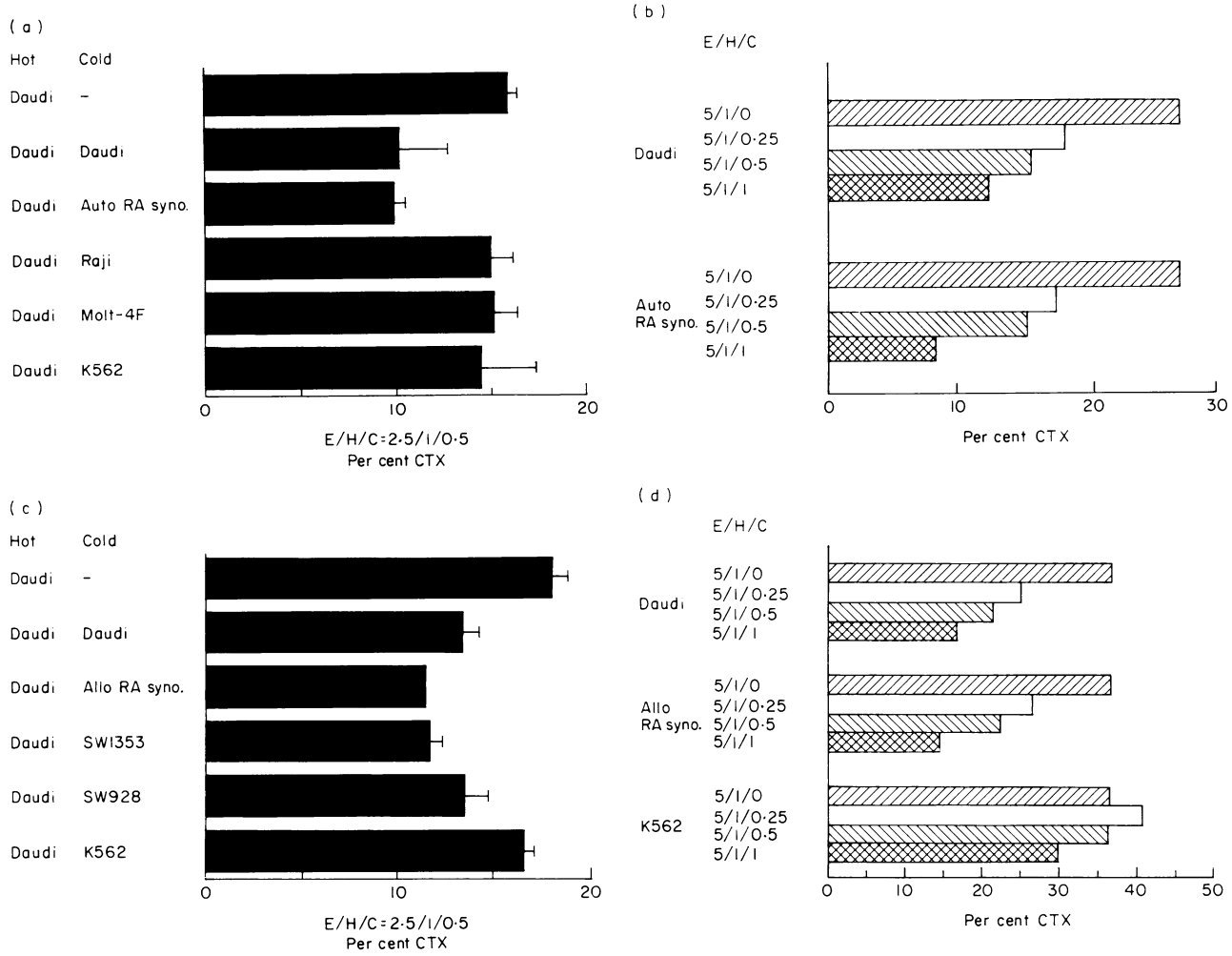

Fig. 6. The cold target inhibition assays of the cytotoxicity (CTX) of rheumatoid arthritis synovial fluid (RASF) TCR Vδ1⁺ T cell line. (a) Cold (^{51}Cr-unlabelled) target cells, such as primarily cultured autologous RA synovial cells, Daudi, Raji, Molt-4F and K562 cell lines, were added to the cytotoxicity assay of RASF Vδ1⁺ T cells against hot (^{51}Cr-labelled) Daudi cells. In this experiment, effectors/hot targets/cold targets (E/H/C) was set at 2·5/1/0·5. The cytotoxicity assay was performed for 5 h. (b) Dose-response study in the cytotoxicity of cold Daudi and primarily cultured RA synovial target cells at different E/H/C ratios. The cytotoxicity assay was performed for 5 h. (c) Cold target inhibition study of the cytotoxicity by using cold allogeneic primarily cultured RA synovial cells, synovial sarcoma (SW928) and chondrosarcoma (SW1353) lines. E/H/C was set at 2·5/1/0·5, and the cytotoxicity was performed for 5 h. (d) Dose-response study of the cytotoxicity of cold allogeneic primarily cultured RA synovial cells. The cytotoxicity was performed for 5 h. Bars represent ± s.e.m.

none of these clones expressed Vδ1 [1]. In rat adjuvant arthritis, the mycobacterial hsp-reactive γδ T cells were suggested to cross-react to the proteoglycans in their cartilage matrix [23]. While mycobacterial hsp65-reactive TCR αβ⁺ CD4⁺ T cell clones derived from RASF were also described in humans [24–26], it may be that certain γδ T cells are also involved in the pathogenesis of human RA. However, the exact role of these T cells in RA development remains to be elucidated.

In general, activated T cells which infiltrate the synovium and synovial fluid are thought to play a significant role in the development of RA. Several cell adhesion molecules may be important for the pathogenesis of RA [27,28]. However, it seems that the selective and clonal expansion of T cells reacting to a certain antigen(s) may occur in RASF. The antigen is supposed to be expressed selectively in the RA synovial tissue. In fact, enrichment of Vδ1⁺ T cells in the RA synovium and synovial fluid was described by some authors, but the etiological and immunobiological role in RA of these T cells has not been demonstrated. It is especially important to know if these T cells could be cytotoxic to the autologous and allogeneic RA synovial cells, since their cytotoxic function may directly explain the destruction of synovial tissue in RA.

Our present study was therefore intended, first, to determine whether the selective expansion of Vδ1⁺ T cells was reproducibly detected in RASF. Second, we assessed if these particular T cells could be cytotoxic to the cells that compose RA synovial tissues.

The data suggested that RASF lines phenotypically showed TCR Vδ1⁺, CD3⁺, CD4⁻ and CD8⁻ T cells. However, the PBL line, which was cultured under the same conditions as RASF, did not express Vδ1, implying that Vδ1⁺ T cells in RA synovium and synovial fluid had already been activated somehow *in vivo*, and been clonally expanded to a certain extent. Furthermore, the data clearly indicated that RASF Vδ1⁺ T

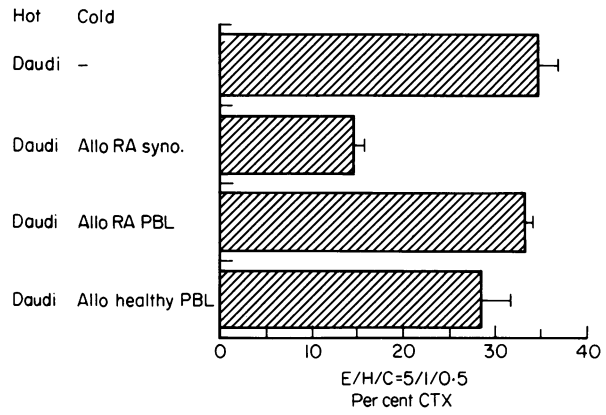

Fig. 7. The selective inhibition by the cold rheumatoid arthritis synovial cells (RASF) of RASF TCR Vδ1$^+$ T cell cytotoxicity (CTX) against Daudi cells. Cold targets, such as primarily cultured RA synovial cells and peripheral blood lymphocytes (PBL) from the same allogeneic RA patient, and PBL from a healthy volunteer, were added to the cytotoxicity assay at E/H/C ratio of 5/1/0·5. The cytotoxicity assay was performed for 5 h. The bars represent ± s.e.m.

cells could be highly cytotoxic to Daudi cells. This cytotoxic response was inhibited, although not completely, by anti-CD3 MoAb, suggesting that TCR Vδ1 molecule may be used to recognize the target Daudi cells. MHC class I and class II molecules may perhaps not be involved in this cytotoxicity. These cellular cytotoxic mechanisms seem to be more general in RA patients, since case 2 showed a similar cytotoxic profile (data not shown).

The cold target inhibition study indicated that both cold autologous and allogeneic primarily cultured RA synovial cells inhibited the cytotoxicity of RASF Vδ1$^+$ T cells against Daudi cells. Allogeneic synovial sarcoma and chondrosarcoma lines also inhibited the cytotoxicity. However, PBL from an allogeneic RA patient was not inhibitory. Moreover, RASF Vδ1$^+$ T cells could directly lyse a SW928 synovial sarcoma line. Taken together, these data strongly suggest that RASF Vδ1$^+$ T cells may recognize a certain antigen(s) that is commonly expressed in the RA synovial tissue. This antigen may also be expressed on Daudi cells, but not on PBL. It is also likely that this antigen may be expressed on the surface of currently employed synovial sarcoma and chondrosarcoma lines.

In our present study, it was unlikely that natural killer (NK) and/or LAK activities were involved, because all of the effectors were CD3$^+$ and did not express NK markers such as CD16 and CD56. Obviously, they also did not lyse K562. Since Vδ1$^+$ T cells are usually only a minor population among PBL, it is considered that they are not the major effector population of LAK cells, either. Although we did not examine the effect of synoviocytes or synovial sarcoma lines in simple NK or LAK assays using K562 targets, our present data strongly suggest an implication in the specific lysis of Vδ1$^+$ T cells against synovial cells.

Meanwhile, immunohistochemical examination of the rheumatoid synovial membrane showed only a few, scattered TCR Vδ1$^+$ T cells [6]. However, the development of RA is supposed to be a complicated disease process, and the T cell populations in the rheumatoid synovial tissue may change, depending upon the phase of the disease process. Our present cytotoxicity data and the fact that TCR Vδ1$^+$ T cells may dominate, at least compared with TCR Vδ2$^+$ T cells, in RA [4–8] strongly suggest that TCR Vδ1$^+$ T cells are involved in RA development.

Recently, the selective recognition of Daudi cells by Vγ9/Vδ2 T cells was reported, and their ligand was described to be human 60-kD hsp expressed on the surface of Daudi cells [29,30]. In the rat model, we demonstrated that cytotoxic CD4$^-$CD8$^-$TCR $\alpha\beta^-$ T cells (presumably $\gamma\delta$ T cells) interacted with a homologue of 70-kD heat shock cognate protein that is expressed on the H-ras oncogene-transformed rat fibroblast [31]. In addition to these hsp as ligand candidates for $\gamma\delta$ T cells, CD1b molecule may participate in the interaction with $\gamma\delta$ T cells [32]. Thus, the immunobiological nature of ligands for $\gamma\delta$ T cells would be diverse. However, the reactivity of RASF Vδ1$^+$ T cells was selective, implying the presence of a specific ligand. In the present study, although we cannot demonstrate the nature of the ligand recognized by RASF Vδ1$^+$ T cells, we are now investigating this important issue by developing several MoAbs which block the cytotoxicity of these particular T cells.

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REFERENCES

1 Holoshitz J, Koning F, Coligan JE *et al.* Isolation of CD4$^-$CD8$^-$ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. Nature 1989; **339**:226–9.

2 Söderström K, Halapi E, Nilsson E *et al.* Synovial cells responding to a 65-kDa Mycobacterial heat shock protein have a high proportion of a TcR$\gamma\delta$ subtype uncommon in peripheral blood. Scand J Immunol 1990; **32**:503–15.

3 Winfield JB. Stress protein, arthritis, and autoimmunity. Arthritis Rheum 1989; **32**:1497–504.

4 Sioud M, Kjeldsen-Kragh J, Quayle A *et al.* The Vδ gene usage by freshly isolated T lymphocytes from synovial fluid in rheumatoid synovitis: a preliminary report. Scand J Immunol 1990; **31**:415–21.

5 Smith MD, Bröker B, Moretta L *et al.* T $\gamma\delta$ cells and their subsets in blood and synovial tissue from rheumatoid arthritis patient. Scand J Immunol 1990; **32**:585–93.

6 Shen Y, Li S, Quayle J *et al.* TCRγ/δ^+ cell subsets in the synovial membranes of patients with rheumatoid arthritis and juvenile rheumatoid arthritis. Scand J Immunol 1992; **36**:533-40.

7 Keystone EC, Rittershaus C, Wood N *et al.* Elevation of a $\gamma\delta$ T cell subset in peripheral blood and synovial fluids of patients with rheumatoid arthritis. Clin Exp Immunol 1991; **84**:78–82.

8 Bucht A, Söderström K, Hultman T *et al.* T cell receptor diversity and activation markers in the Vδ1 subset of rheumatoid synovial fluid and peripheral blood T lymphocytes. Eur J Immunol 1992; **22**:567–74.

9 Halstensen TS, Scott H, Brandtzaeg P. Intraepithelial T cells of the TcRγ/δ^+CD8$^-$ and Vδ1/Jδ1$^+$ phenotypes are increased in coeliac disease. Scand J Immunol 1989; **30**:665–72.

10 Autrun B, Triebel F, Katlama C *et al.* T cell receptor γ/δ^+ lymphocyte subsets during HIV infection. Clin Exp Immunol 1989; **75**:206–10.
11 Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; **31**:315–24.
12 Ishii Y, Kon S, Fujimoto J, Kikuchi K. Four distinct antigen systems on human thymus and T cells defined by monoclonal antibodies: immunohistrogical and immunochemical studies. Clin Exp Immunol 1983; **53**:31–39.
13 Sato T, Sato N, Takahashi S *et al.* Specific cytotoxicity of a long-term cultured T-cell clone on human autologous mammary cancer cells. Cancer Res 1986; **46**: 4384–9.
14 Wada Y, Ikeda H, Ueda D, Ohta M *et al. In vitro* proliferation and cytotoxic specificity of a cryopreserved cytotoxic T cell clone reacting against human autologous tumor cells. J Immunol Methods 1992; **154**:235–43.
15 Casorati G, Libero GD, Lanzavecchia A, Migone N. Molecular analysis of human γ/δ^+ clones from thymus and peripheral blood. J Exp Med 1989; **170**:1521–35.
16 Holoshitz J, Vila LM, Keroack BJ *et al.* Dual antigen recognition by cloned human γδ T cells. J Clin Invest 1992; **89**:308–14.
17 Happ MP, Kubo RT, Palmer E *et al.* Limited receptor repertoire in a mycobacteria-reactive subset of γδ T lymphocytes. Nature 1989; **342**:696–8.
18 Ohmen JD, Branes PF, Uyemura K *et al.* The T cell receptors of human γδ T cells reactive to *Mycobacterium tuberculosis* are encoded by specific V genes but diverse V–J junctions. J Immunol 1991; **147**:3353–9.
19 Janis EM, Kaufmann SHE, Schwartz RH, Pardoll DM. Activation of γδ T cells in the primary immune response to *Mycobacterium tuberculosis*. Science 1989; **244**:713–6.
20 O'Brien RL, Happ MP, Dallas A *et al.* Stimulation of a major subset of lymphocytes expressing T cell receptor γδ by an antigen derived from *Mycobacterium tuberculosis*. Cell 1989; **57**:667–74.
21 Haregewoin A, Soman G, Hom RC, Finberg RW. Human $\gamma\delta^+$ T cells respond to mycobacterial heat-shock protein. Nature 1989; **340**:309–12.
22 Born W, Hall L, Dallas A *et al.* Recognition of a peptide antigen by heat shock-reactive γδ T lymphocytes. Science 1990; **249**:67–69.
23 van Eden W, Thole JER, van der Zee R *et al.* Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 1988; **331**:171–3.
24 Gaston JSF, Life PF, van der Zee R *et al.* Epitope specificity and MHC restriction of rheumatoid arthritis synovial T cell clones which recognize a mycobacterial 65 kDa heat shock protein. Int Immunol 1991; **3**:965–72.
25 Gaston JHS, Life PF, Jenner PJ *et al.* Recognition of a mycobacteria-specific epitope in the 65-kD heat-shock protein by synovial fluid-derived T cell clones. J Exp Med 1990; **171**:831–41.
26 Gaston JHS, Life PF, Bailey LC *et al. In vitro* responses to a 65-kilodalton mycobacterial protein by synovial T cells from inflammatory arthritis patients. J Immunol 1989; **143**:2494–500.
27 Iigo Y, Takashi T, Tamatani T *et al.* ICAM-1-dependent pathway is critically involved in the pathogenesis of adjuvant arthritis in rats. J Immunol 1991; **147**:4167–71.
28 van Dinther-Janssen ACHM, Horst E, Koopman G *et al.* The VLA-4/VCAM-1 pathway is involved in lymphocyte adhension to endothelium in rheumatoid synovium. J Immunol 1991; **147**:4207–10.
29 Fisch P, Malkovsky M, Kovats S *et al.* Recognition by human Vδ9/Vδ2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. Science 1990; **250**:1269–72.
30 Kaur I, Voss SD, Gupta RS *et al.* Human peripheral γδ T cells recognize hsp60 molecules on Daudi Burkitt's lymphoma cells. J Immunol 1993; **150**:2046–55.
31 Tamura Y, Tsuboi N, Sato N, Kikuchi K. 70 kDa heat shock cognate protein is a transformation-associated antigen and a possible target for the host's anti-tumor immunity. J Immunol 1993; **151**:5516–24.
32 Porcelli S, Morita CT, Brenner MB. CD1b restricts the response of human $CD4^-8^-$ T lymphocytes to a microbial antigen. Nature 1992; **360**:593–7.