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Recognition of the Qa-2^k tumor antigen by T cell receptor γ/δ of an immunopotentiator-induced tumoricidal T cell of mice

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Abstract. Tumor-specific expression of Qa-2^k antigen coded by the Q5^k gene on various mouse tumor cells and immunological response of the host mice to the antigen have been demonstrated [Seo et al. (1992) J Exp Med 175: 547; Tanino et al. (1992) Cancer Immunol Immunother 35: 230]. The possibility was examined that Qa-2 antigen is one of the recognition target molecules of immunopotentiator-induced, H-2-nonrestricted tumoricidal lymphocytes of Qa-2$^-$ mice. Lymphocytes stimulated in vivo with *P. acnes* or culture-induced anomalous killers of B6.K1 mice did not exhibit significant in vitro cytotoxicity against B6.K1 lymphoblasts but lysed their Qa-2,3-congenic counterpart B6 lymphoblasts. To demonstrate the Qa-2 specificity of such cytotoxic cells more precisely, an L cell transformant clone (L$^{Q7b/Kb}$), which expressed the α1 and α2 domains of the Qa-2 antigen (Q7^b gene product), was generated by transfecting a cloned plasmid DNA containing a hybrid gene constructed from the 5′ half of the Q7^b gene and the 3′ half of the H-2K^b gene (pQ7^b/K^b). Using L$^{Q7b/Kb}$ cells as the target cells and the nylon-wool-nonadherent fraction of lymphocytes from *P. acnes*-stimulated (C3H/He × B6.K1)F1 mice (H-2^k, Qa-2$^-$) as the effector cells of the in vitro cytotoxicity reaction, the presence of cytotoxic cells that recognize the α1/α2 region of the Q7^b gene product was demonstrated. The cytotoxic activity was dependent on T cells bearing T cell receptors of the γ/δ type (TCRγ/δ). The (C3H/He × B6.K1)F1 effector cells, as well as the B6.K1 effector cells also lysed BW5147 lymphoma cells (Qa-2^{k+}) derived from AKR mice (Qa-2$^-$, H-2^k). By target-competition experiments it was shown that some of the effector cells lytic to BW5147 were identical to those that lysed L$^{Q7b/Kb}$. Therefore some of the tumoricidal cells induced by the immunopotentiator interact with the target tumor cells through recognition of the α1/α2 region of the Qa-2^k tumor antigen by TCRγ/δ.

Key words: Qa-2 – TCRγ/δ – Anomalous killer – Immunopotentiator

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Introduction

A group of lymphocytes are known to have major histocompatibility-complex(MHC)-nonrestricted inherent cytotoxic activity against tumor cells. A number of them, including natural killer (NK) cells, lack the T cell receptor (TCR) and are thought to bind target tumor cells by interactions between cell-adhesion molecules such as LFA-1 and ICAM-1 [7, 16]. Another subset seems to recognize target cells using the TCR. Some of the cells in the latter group have been cloned and reported to be CD3+4−8− or CD3+4−8+ lymphocytes [9, 10]. Unlike NK cells, most of these cells seem to be present in non-cytotoxic forms in normal animals and can be activated in vivo by stimulations with immunopotentiators such as *Bacillus Calmette-Guerin* or *Propionibacterium acnes* [5], or in vitro by culturing lymphocytes in the presence of fetal calf serum [13] or lymphokines [10, 20]. Such antigen-nonspecifically induced cytotoxic cells generally lyse target tumor cells in an MHC-nonrestricted manner and affect syngeneic normal cells to a less extent. To understand the mechanism by which such cytotoxic cells discriminate malignant cells from normal cells, it is important to clarify the recognition target molecules of their TCR.

In a preceding report [23], we demonstrated a possibility that allogeneic lymphocyte antigens including those of the Qa, TL and Ly groups, expressed illegitimately on tumor cells, are recognition targets of the immune systems of the syngeneic host mice. Among them, the Qa-2 antigen expressed on tumor cells was proved to be a recognition target of one of the antibodies of the IgD class detected in the serum obtained from mice after regression of the syngeneic tumor cells without specific immunization. Expression of the Qa-2 antigen was detected on the surface of cells of most of the tumor cell lines derived from Qa-2$^-$ mice. The Qa-2 antigen is a serologically defined nonclassical histocompatibility class 1 alloantigen of mice coded by a nonpolymorphic class 1 antigen gene and is known to be selectively expressed on lymphocytes [24]. The extracellular portion of the Qa-2 molecule, like those of all other histocompatibility class 1 antigen molecules, is composed

of two regions: the region constructed from the α1 and the α2 domains and the region composed of the α3 domain. The former carries the major part of the Qa-2 specificity while the latter is highly homologous to other class 1 antigens. We showed recently that the Qa-2 antigen expressed on tumor cells derived from Qa-2$^-$ H-2^k mice (Qa-2^k antigen) is distinct from the Qa-2 antigen on the normal lymphocyte surface of Qa-2$^+$ mice in some respects, although the two antigens are similar to each other and cross-react with some Qa-2-specific monoclonal antibodies (mAb) and with the IgD in the tumor-regressor serum [21, 23]. The former was shown to be the product of the Q5^k gene [21] while the latter is coded by the Q7 gene [26]. Although it was difficult to analyze directly the recognition target molecules of the immunopotentiator-induced cytotoxic cells mentioned above, these results made us consider the possibility that the products of the Q7 and Q5 genes were recognized by TCR of such cells. This possibility is studied in the present communication.

Materials and methods

Animals, tumor cells, monoclonal antibodies and complement

C57BL/6 (B6), B6.K1, C3H/He and (C3H/He × B6.K1)F1 mice were used. B6 and B6.K1 are congenic with respect to Qa-1, Qa-2 and Qa-3 antigens (B6; Qa-1$^-$2$^+$3$^+$ and B6.K1; Qa-1$^+$2$^-$3$^-$) [22]. BW5147, an in vitro lymphoma cell line derived from AKR mice (H-2^k, Qa-2$^-$), was cultured in RPMI-1640 medium supplemented with 5% fetal calf serum (FCS). Expression of the Qa-2 antigen (Q5^k gene product) on BW5147 cells has been demonstrated [21, 23]. Qa-2-specific monoclonal antibody (mAb) 34-1.2, which recognizes the α1/α2 region of the Qa-2 antigen, was obtained from American Type Culture Collection, Mass. A hamster mAb specific to mouse TCR of the α/β type (TCRα/β-specific mAb 57.597,2.5) [15] and one specific to mouse TCR of the γ/δ type (TCRγ/δ-specific mAb 903A10) [12] were purchased from PharMingen, Calif. A mAb specific to a bacterial component (*E. coli* NusA protein) was a gift from Dr. Y. Nakamura of this laboratory and was used as a mAb not related to mouse components.

Effector cells and assay of cytotoxicity

Anti Qa-2,3 cytotoxic T lymphocytes (CTL) were induced by immunizing male B6.K1 mice 7 or 8 weeks old by repeated intraperitoneal inoculation of 1×10^7 B6 lymphocytes (mixture of splenocytes and mesenteric lymph node cells). The splenocytes obtained from the immunized mice were stimulated in vitro by culturing for 5 days at a cell density of 1×10^7 cells/ml in the presence of mitomycin-C-treated B6 lymphocytes at a concentration of 3×10^6 cells/ml. The resulting cells were washed with the medium and used as the effector cells in cytotoxicity assays. Anti-H-2^k CTL were similarly induced using B6 mice as the responder and C3H/He lymphocytes as the immunogen and the stimulator. Cytotoxic activity was also induced either in vivo or in vitro without specific antigenic stimulation. Splenocytes obtained from normal B6.K1 mice were cultured for 3 days in RPM1-1640 medium supplemented with 10% FCS and 50 μM 2-mercaptoethanol at a cell density of 4×10^6 in 2 ml/well using 12-well plates (Flow Laboratories, Inc., McLean, Va.). Dish-nonadherent cells were obtained after culturing and were used as the effector cells of culture-induced anomalous cytotoxicity [13]. Lymphokine-activated killers (LAK) were obtained by culturing splenocytes from normal B6.K1 or C3H/He mice in the same way as above in RPMI-1640 medium supplemented with 10% FCS, 50 μM 2-mercaptoethanol and 10% culture supernatant, which was obtained from a 2-day culture of rat splenocytes (2×10^7 cells/ml) in the presence of 10% FCS and 5 μg/ml concanavalin A. Immunopotentiator-induced cytotoxic lymphocytes were obtained by administering 1 mg heat-killed *P. acnes* (strain IID912 provided by the Laboratory of Culture Collection of this institute) to the peritoneal cavity of a mouse. The nylon-wool-nonadherent fraction of the splenocytes obtained 3 days afterwards was used as the effector cells of the cytotoxicity reactions [5].

Cytotoxic activity of the effector cells was assayed by incubating 1×10^4 ^{51}Cr-labelled target cells with various numbers of effector cells in 0.4 ml RPMI-1640 medium supplemented with 7% FCS at 37° C in a CO_2 incubator for 5–10 h. After the incubation, the radioactivity released into the supernatant and that remaining in the cells was measured and the percentage specific lysis was calculated as previously described [13]. Lymphoblasts, BW5147 cells and the L cell transformants generated as described below were used as the target cells. Lymphoblasts were prepared by culturing normal mouse splenocytes at a concentration of 5×10^5 cells/ml for 2 days in RPM1-1640 medium supplemented with 10% FCS and 2-mercaptoethanol and then for 3 days in the medium containing 5 μg/ml concanavalin A.

Construction of the pQ7^b/K^b plasmid and generation of L cell transformant clones

A plasmid clone Q7^{b}pTCF [26], which consists of an approximately 11×10^3-base (11 kb) *Hin*dIII fragment of a cosmid clone B2.17 subcloned into pTCF and comprising the entire Q7^b gene and an approximately 3-kb flanking sequence at each end, was generously provided by Dr. R. A. Flavell (Biogen Research Corp., Cambridge). A *Pst*I fragment of about 5 kb, containing the H-2K^b gene, was cloned into pBR322 and used as a plasmid clone pK^{b}3-pst-2. A plasmid pOPF1 contains the herpes simplex virus thymidine kinase (*tk*) gene. A 5.8-kb *Hin*dIII-*Bam*HI fragment containing exons 1–3 of the Q7^b gene was obtained from Q7^{b}pTCF and cloned into the *Hin*dIII and *Bam*HI sites of pBR322. From this plasmid, an almost 3.2-kb *Bgl*II-*Bam*HI fragment containing exons 1–3 of the Q7^b gene was prepared. This fragment was cloned into the *Bgl*II and *Bam*HI sites of pUC19(*Bgl*II). A 2.3-kb *Bam*HI fragment containing exons 4–8 of the H-2K^b gene was obtained from pK^{b}3-pst-2 and cloned into the *Bam*HI site of this plasmid. A plasmid clone that contained exons 1–3 of Q7^b and exons 4–8 of H-2K^b in the right orientation was selected (pQ7^b/K^b).

L^{tk-} cells were transformed by DNA transfection using the calcium phosphate coprecipitation method [27]. pQ7^b/K^b or pK^{b}3-pst-2 DNA (20 μg) was mixed with 0.25 μg pOF1 DNA and the DNA was precipitated with 0.15 M calcium chloride. The precipitate was placed on about 1×10^7 confluent L^{tk-} cells. After culturing for 4 h in Dulbecco's modified minimal essential medium (DMEM), the cells were washed with the same medium, treated for 15 with 30% glycerol, washed again with DMEM and then cultured in DMEM containing 100 μM hypoxanthine, 0.4 μM aminopterin and 16 μM thymidine (HAT). The HAT-resistant cells (1×10^4) were plated on a 75-cm^2 culture flask and cultured for 5–6 days and the resulting isolated colonies were transfered to 12-well plates using filter-paper disks of 3 mm diameter. A colony that strongly expressed either the Qa-2 or the H-2K^b phenotype was selected (L$^{Q7b/Kb}$ or L^{Kb} respectively) and used as target cells of the cytotoxicity reactions. Detection of the Qa-2 and H-2K^b phenotypes was carried out as described elesewhere [23] using anti-Qa-2 mAb 34-1.2 and anti-H-2K^b mAb. The transformants also expressed the H-2^k phenotype derived from the original L^{tk-} cells together with the Qa-2 or the H-2K^b phenotype.

Results

Lysis of B6 lymphoblasts by antigen-nonspecifically induced cytotoxic lymphocytes of B6.K1 mice

Anomalous killers were induced by culturing normal B6.K1 spleen cells in vitro without specific antigenic stim-

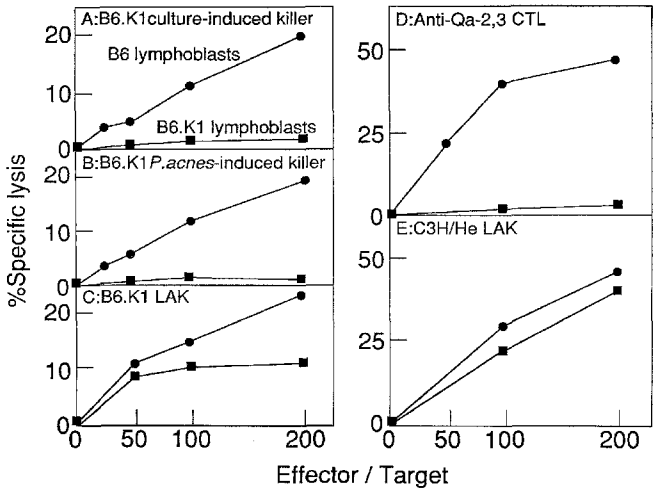

Fig. 1 A–E. Qa-2,3-specific lytic activity of antigen-nonspecifically induced killers of B6.K1. Lysis of B6 (●) and B6.K1 (■) lymphoblasts was assayed using various cytotoxic cells. **A** Culture-induced anomalous killer of B6.K1; **B** *P. acnes*-induced cytotoxic lymphocytes of B6.K1; **C** B6.K1 lymphokine-activated killer cells (*LAK*); **D** anti-Qa-2,3 cytotoxic T lymphocytes (*CTL*); **E** C3H/He LAK. Incubations were carried out at 37° C for 5–6 h. Values show means ± SE of the results of two experiments

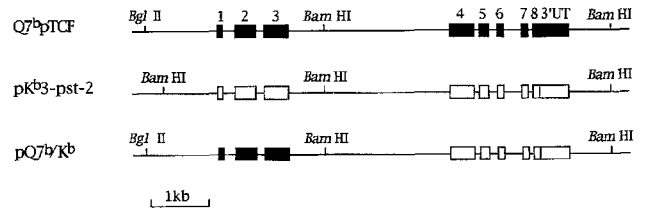

Fig. 2. Exon/intron structures of constructs Q7^{b}pTCF, pK^{b}3-pst-2 and pQ7^{b}K^b. The Q7^b exons and 3′ untranslated region (*3′UT*) are indicated by *shaded boxes*; H-2K^b exons and 3′UT by *open boxes*; introns and 5′ and 3′ flanking sequences by *solid lines*

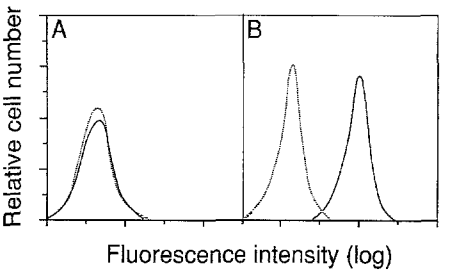

Fig. 3 A, B. Detection of Qa-2 specificity on L$^{Q7b/Kb}$ cells by flow cytometry. **A** L^{tk-} cells; **B** L$^{Q7b/Kb}$ cells. Anti-Qa-2 mAb 34-1.2 (———) or non-related mAb (...) were used as the first antibody and fluorescein-isothiocyanate-labelled F(ab′)$_2$ of anti-(mouse immunoglobulin) goat antibody was used as the second antibody

ulation and without addition of lymphokines. They lysed the Qa-2,3 congenic counterpart B6 lymphoblasts to some extent but not syngeneic B6.K1 lymphoblasts (Fig. 1 A). A similar result was obtained when lymphocytes from B6.K1 mice pretreated with *P. acnes* were used as the effector cells (Fig. 1 B). Although only limited activity was observed in these cases, the activity was significant and reproducible. When LAK cells were induced using B6.K1 lymphocytes and used as the effectors, they lysed B6.K1 lymphoblasts to some extent and B6 lymphoblasts considerably more strongly (Fig. 1 C). In control experiments, anti-Qa-2 CTL lysed B6 lymphoblasts strongly without affecting B6.K1 lymphoblasts (Fig. 1 D) whereas no significant difference was observed between lysis of these lymphoblasts by LAK induced using C3H/He lymphocytes (Fig. 1 E). All of these effector cells more or less lysed Qa-2+ tumor cells such as BW5147, MM2, MH134 and EL-4 (data not shown). These results seem to show that, at least in the *P. acnes*-stimulated lymphocytes and in the culture-induced anomalous killers, there is a population of cytotoxic cells that recognize Qa-2 or Qa-3 antigen on the surfaces of the target cells.

Lysis of L$^{Q7b/Kb}$ and BW5147 cells by P. acnes-stimulated (C3H/He × B6.K1)F1 lymphocytes

It has been reported that the Q7^b gene in the Qa/Tla gene region of C57BL/10 (H-2^b) mice codes for serologically defined Qa-2 antigen and that a hybrid gene constructed from the 5′ half (containing exons 1, 2 and 3, which code for the leader sequence, the α1 domain and the α2 domain respectively) of the Q7^b gene and the 3′ half (containing exons 4–8, which code for the α3 domain, the transmembrane region and the cytoplasmic region) of an H-2 gene, when transfected into cells of non-lymphocyte origin, can code for a cell-surface molecule with Qa-2 specificity [26]. For these reasons, we constructed a hybrid gene from the 5′ half of the Q7^b gene and the 3′ half of the H-2K^b gene as shown in Fig. 2, transfected it into L^{tk-} cells derived from C3H/He mice (H-2^k, Qa-2$^-$) and a clone strongly expressing the Qa-2 phenotype was obtained (Fig. 3). The cell-surface antigen molecule is composed of α1 and α2 domains of the Qa-2 molecule, and α3, transmembrane and cytoplasmic domains of the H-2K^b molecule. An L cell transformant clone expressing the H-2K^b whole molecule was similarly generated by transfecting pK^{b}3-pst-2 DNA. The cytotoxic activity of lymphocytes obtained from (C3H/He × B6.K1)F1 mice (H-2$^{k/b}$, Qa-2$^-$) pretreated with *P. acnes* was tested using L$^{Q7b/Kb}$ and L^{Kb} cells as the target cells (Fig. 4). L$^{Q7b/Kb}$ cells were susceptible to the lytic activity of the *P. acnes*-stimulated lymphocytes. The activity was limited but nevertheless significant and reproducible. The effector activity of the lymphocytes was mainly associated with their nylon-wool-nonadherent fraction. The same effector cells did not lyse nontransfected L^{tk-} cells nor L^{Kb} cells. Also, none of these target cells was lysed by spleen cells obtained from normal mice or their nylon-wool-nonadherent fraction. On the other hand, these target cells were lysed to a similar extent by the anti-H-2^k CTL, showing that the specific sensitivity of L$^{Q7b/Kb}$ to the *P. acnes*-stimulated lymphocytes was not due to possiblly enhanced lability of the cell line compared to others. These results indicate that nylon-wool-nonadherent lymphocytes obtained from Qa-2$^-$ (C3H/He × B6.K1)F1 mice pretreated with

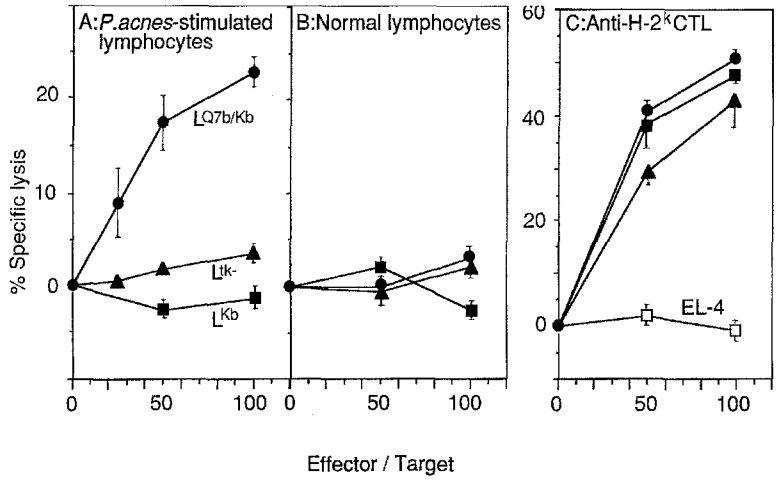

Fig. 4 A – C. Qa-2-specific cytotoxic activity of *P. acnes*-stimulated lymphocytes of (C3H/He × B6.K1)F1 mice. Nylon-wool-nonadherent fraction of splenocytes from the F1 mice after in vivo stimulation with *P. acnes* (**A**), those from the normal F1 mice (**B**) and anti-H-2^k CTL (**C**) were used as the effector cells. Target cells: L^{tk-} cells (▲), L$^{Q7b/Kb}$ cells (●), L^{Kb} cells (■) and EL-4 (H-2^b) cells (□). Incubations were carried out at 38° C for 10 h. Values show means ± SE of the results of two experiments

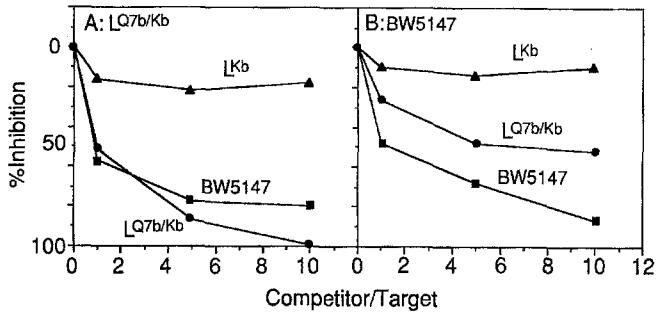

Fig. 5 A, B. Target competition between BW5147 and L$^{Q7b/Kb}$ cells. Nylon-wool-nonadherent fraction of splenocytes from *P. acnes*-stimulated (C3H/He × B6.K1)F1 mice were used as the effector cells. The reaction mixture contained 1×10^4 ^{51}Cr-labelled target cells, 4×10^5 effector cells and various amounts of nonlabelled competitor cells in 0.4 ml medium. Incubations were carried out at 37° C for 6 h. The percentage specific lysis of L$^{Q7b/Kb}$ and BW5147 cells in the absence of competitor cells was 30.6±0.9% and 53.3±0.8% respectively. Target cells: **A** L$^{Q7b/Kb}$; **B** BW5147. Competitor cells: L$^{Q7b/Kb}$ (●), BW5147 (■) and L^{Kb} (▲)

P. acnes contained cytotoxic cells that interact with the target cells through recognition of the α1/α2 region of the Q7^b gene product.

The effector cells induced by *P. acnes* were lytic not only to L$^{Q7b/Kb}$ cells but also to BW5147 tumor cells. BW5147 cells were derived from AKR (H-2^k, Qa-2$^-$) mice and the Qa-2 antigen was detected on their cell surfaces [23]. However, while the present study was in progress, it became clear that the Qa-2 antigen expressed illegitimately on tumor cells is the Q5 gene product whereas the Qa-2 antigen of normal lymphocytes is the Q7 gene product and these two molecules are distinct from each other in some respects [21]. Therefore there was a possibility that lymphocytes that were cytotoxic to BW5147 cells were different from those that lysed L$^{Q7b/Kb}$. To test this possibility, target competition experiments were carried out using BW5147 and L$^{Q7b/Kb}$ cells as the target and the competitor cells (Fig. 5). Lysis of L$^{Q7b/Kb}$ cells by the *P. acnes*-stimulated cells showed complete competition by BW5147 cells and the lysis of BW5147 showed partial competition by L$^{Q7b/Kb}$. L^{Kb} cells competed in neither lysis. These results showed that a some of the cells lytic to BW5147 were identical to those lytic to L$^{Q7b/Kb}$. It was also found that the cytotoxic activity of *P. acnes*-induced effectors to lyse L$^{Q7b/Kb}$ cells decreased when the cells were treated with with TCRγ/δ-specific mAb and complement but not when they were treated with TCRα/β-specific mAb and complement (Table 1). Therefore it seems that the tumoricidal cells induced by the administration of immunopotentiating agents such as *P. acnes* to Qa-2$^-$ mice contained cytotoxic T cells bearing the TCRγ/δ with specificity for the α1/α2 regions of the Qa-2 antigens of both tumor cells and normal lymphocytes.

Table 1. T cell receptor (TCR) of the *P. acnes*-induced (C3H/He × B6.K1)F1 cytotoxic cells[a]

Treatment	Specific lysis (%)
None (complement only)	19.8±0.9
TCRα/β-specific mAB + complement	20.6±1.1
TCRγ/δ-specific mAb + complement	1.8±0.1

[a] The assay mixture contained in 0.4 ml 1×10^4 ^{51}Cr-labelled L$^{Q7b/Kb}$ cells and effector cells obtained after the treatment of 1×10^6 nylon-wool-nonadherent *P. acnes*-induced cells. Incubation was carried out at 37° C for 6 h. Values show means ± SE of the results of two experiments

Discussion

The physiological roles of non-classical class I histocompatibility antigens coded by genes in the Qa/Tla region are not known. We reported previously that the Qa-2 antigen is expressed on the surface of various tumor cells derived from Qa-2$^-$ mice and is one of the recognition targets of immunoglobulins of the IgD class produced in response to the tumor cells by the host mice [23]. In the present report, we demonstrated the presence of T cells in Qa-2$^-$ mice that could be activated by an antigen-nonspecific stimulation and exerted tumoricidal activity through recognition of the Qa-2 antigen expressed on the tumor cell surface. We have also reported that the Qa-2 antigen expressed on tumor cells derived from Qa-2$^-$ H-2^k mice is coded by the the Q5^k gene, while the Qa-2 antigen on normal H-2^b lymphocytes

is a $Q7^b$ or $Q9^b$ gene product [21]. The present report also shows that *P. acnes*-stimulated lymphocytes of the Qa-2$^-$ mice contained a population of cytotoxic T cells that seem to recognize a structure common to both the $Q5^k$ and $Q7^b$ gene products. Such cytotoxic cells may not be induced in Qa-2$^+$ mice. However, the Q5 gene product was also detected on the surface of tumor cells such as EL-4, which is a T lymphoma cell line derived from B6 (Qa-2$^+$) mice (unpublished result). Therefore, Q5-specific recognition may also take part in the immunopotentiator-induced resistance against tumors in Qa-2$^+$ mice. The Qa-2 antigen may be one of the multiple recognition targets of the antigen-nonspecifically activated cytotoxic T lymphocytes. Some of other allogeneic (alien) lymphocyte antigens expressed on tumor cells, such as those of the TL and Ly groups, may also be their direct recognition targets.

Physiological contributions of T cells bearing TCRγ/δ to immune responses is not clear. As for ligands recognized by TCRγ/δ, it has been shown that γ/δ T cells interact with target cells through MHC-nonrestricted recognition of allogeneic MHC molecules [4, 7, 19], non-classical MHC class 1 antigens such as TL [1, 2, 11], Qa-1 [25] and CD1c [6, 18], and through MHC-restricted recognition of mycobacterial heat-shock proteins [8], tetanus toxoid [14], etc. The MHC-nonrestricted cytotoxicity displayed by γ/δ T cells suggests their possible role as effector cells in "immune surveillance" to eliminate abnormal cells such as tumor cells. The result presented in this study provides evidence that some lymphocytes, in particular of the γ/δ type, interact specifically with another nonclassical MHC class 1 antigen, namely the $Qa\text{-}2^k$ antigen ($Q5^k$ gene product), expressed on the syngeneic tumor cells. Normal allogeneic Qa-2 antigen coded by the $Q7^b$ gene cross-reacted with the effector cells. Since expression of the $Q5^k$ gene has been shown to be tumor-specific and to take place on various tumor cells, and the γ/δ CTL specific to the $Q5^k$ gene product is induced by antigen-nonspecific stimulation, it seems that interaction between such CTL and the $Q5^k$ gene product contributes to inherent immunological resistance against malignancies. Further characteristics of the *P. acnes*-induced cytotoxic cells were not clarified because of the low activity of the cells. We tried to generate Qa-2-specific CTL clones using B6.K1 splenocytes from mice actively immunized with B6 lymphocytes. We obtained several such clones, which were cytotoxic not only to B6 lymphoblasts but also to BW5147 cells. However, they were all found to bear TCRα/β. It seems, therefore, that switch from γ/δ T cells to α/β T cells takes place after specific in vivo immunization. For further characterization of the Qa-2-specific γ/δ CTL, generation of clones of such cells using some other strategy will be necessary.

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References

1. Bluestone JA, Cron RQ, Cotterman M, Houlden BA, Matis LA (1988) Structure and specificity of T cell receptor γ/δ on major histocompatibility complex antigen-specific CD3$^+$, CD4$^-$, CD8$^-$ lymphocytes. J Exp Med 168: 1899
2. Bonneville M, Ito K, Krecko EG, Itohara S, Kappes D, Ishida I, Kanagawa O, Janeway CA, Murphy DB, Tonegawa S (1989) Recognition of a self major histocompatibility complex TL region product by $\gamma\delta$ T cell-receptors. Proc Natl Acad Sci USA 86: 5928
3. Born WK, O'Brien RL, Modlin RL (1991) Antigen specificity of γ/δ lymphocytes. FASEB 5: 2699
4. Ciccone E, Viale O, Bottino C, Pende D, Migone N, Gasorati G, Tambussi G, Moretta A, Moretta L (1988) Antigen recognition by human T cell receptor γ-positive lymphocytes: specific lysis of allogeneic cells after activation in mixed lymphocyte culture. J Exp Med 167: 1517
5. Egawa K, Tanino T (1982) Nonspecific cytotoxic cells induced in vivo and their comparison to culture-induced cytotoxic cells. Jpn J Exp Med 52: 237
6. Faure F, Jitsukawa S, Miossec C, Hercend T (1990) CD1c, as a target structure for human T lymphocytes: analysis with peripheral blood γ/δ cells. Eur J Immunol 20: 703
7. Hall RE (1985) A monoclonal antibody (RH1-38) which inhibits multiple systems of cell mediated cytotoxicity: I. Identification of a novel epitope on a killer cell surface bimolecular complex important in the cytotoxic response. Mol Immunol 22: 757
8. Haregervoin A, Soman G, Hom RC, Finberg RW (1989) Human $\gamma\delta^+$ T cells respond to mycobacterial protein. Nature 340: 309
9. Hera A, Toribio ML, Marquez C, Martinez-A C (1985) Interleukin 2 promotes growth and cytolytic activity in human T3$^+$4$^-$8$^-$ thymocytes. Proc Natl Acad Sci USA 82: 6268
10. Hercend T, Reinherz EL, Meuer S, Schlossmann SF, Ritz J (1983) Phenotypic and fuctional heterogeneity of human cloned natural killer cell lines. Nature 301: 158
11. Ito H, Kaer LV, Bonneville M, Hsu S, Murphy DB, Tonegawa S (1990) Recognition of the product of a novel MHC TL region gene (27^b) by a mouse $\gamma\delta$ T cell receptor. Cell 62: 549
12. Itohara S, Nakanishi N, Kanagawa O, Kubo R, Tonegawa S (1989) Monoclonal antibodies specific for native murine T-cell receptor $\gamma\delta$: Analysis of $\gamma\delta$ T cells during thymic ontogeny and in peripheral lymphoid organs. Proc Natl Acad Sci USA 86: 5094
13. Kiyohara T, Tanino T, and Egawa K (1982) T cell-dependent nonspecific cytotoxicity induced by culture of mouse spleen cells against natural killer-insensitive tumor cells. Jpn J Exp Med 52: 201
14. Kozbor D, Trinchieri G, Monos DS, Isobe M, Russo G, Haney JA, Zimijewski C, Croce CM (1989) Human TCR-γ^+/δ^+, CD8$^+$ T lymphocyte recognize tetanus toxoid in an MHC-restricted fashon. J Exp Med 169: 1847
15. Kubo RT, Born W, Kappler JW, Marrack P, Pigeon M (1989) Characterization of a monoclonal antibody which detects all murine $\alpha\beta$ T cell receptors. J Immunol 142: 2736
16. Markgoba MW, Sanders ME, Ginther Luce GE, Dustin ML, Springer TA, Clark EA, Mannoni P, Shaw S (1988) ICAM-1, a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. Nature 331: 86
17. Matis LA, Fry AM, Cron RQ, Cotterman MM, Dick RF, Bluestone JA (1989) Structure and specificity of a class II MHC alloreactive γ/δ T cell receptor heterodimer. Science 245: 746
18. Terhorst C, Bleicher PA (1989) Recognition of cluster of differentiation 1 antigen by human CD4$^-$CD8$^-$ cytotoxic T lymphocytes. Nature 341: 447
19. Rivas A, Koide J, Cleary ML, Engleman EG (1989) Evidence for involvement of the γ/δ T cell antigen receptor in cytotoxicity mediated by human alloantigen-specific T cell clones. J Immunol 142: 1840
20. Rosenstein M, Yron I, Kaufmann Y, Rosenberg SA (1984) Lymphokine-activated killer cells: lysis of fresh syngeneic natural killer-resistant murine tumor cells by lymphocytes cultured in interleukin 2. Cancer Res 44: 1946

21. Seo N, Okazaki T, Nakanishi-Ito C, Tanino T, Matsudaira Y, Takahashi T, Egawa K (1992) Expression of the Qa-2^k phenotype encoded by the $Q5^k$ gene on the surface of tumor cells derived from H-2^k mice. J Exp Med 175, 547
22. Stanton TH, Boyse EA (1976) A new serologically defined locus, Qa-1, in the Tla-region of the mouse. Immunogenetics 3: 525
23. Tanino T, Seo N, Okazaki T, Nakanishi-Ito C, Sekimata M, Egawa K (1992) Detection of allogeneic Qa/TL and Ly specificities on murine tumor cells with IgD in tumor regressor serum. Cancer Immunol Immunother 35: 230
24. Vernachio J, Li M, Donnenbery AD, Soloski MJ (1989) Qa-2 expression in the adult murine thymus. A unique marker for a thymic subset. J Immunol 142: 48
25. Vidovic D, Roglic M, McKune K, Guerder S, McKay C, Dembic Z (1989) Qa-1 restricted recognition of foreign antigen by γ/δ T cell hybridoma. Nature 340: 646
26. Waneck GL, Sherman DH, Clavin S, Allen H, Flavell RA (1987) Tissue-specific expression of cell-surface Qa-2 antigen from a transfected $Q7^b$ gene of C57BL/10 mice. J Exp Med 165: 1358
27. Wigler M, Pellicer A, Silverstein S, Axel R, Urlaub G, Chasin L (1979) DNA-mediated transfer of the adenine phosphoribosyl transferase locus into mammalian cells. Proc Natl Acad Sci USA. 76: 1373