Brief Definitive Report

Selective Long-Term Elimination of Natural Killer Cells In Vivo by an Anti-interleukin 2 Receptor β Chain Monoclonal Antibody in Mice

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Summary

The interleukin 2 receptor β chain (IL-2Rβ) is preferentially expressed in natural killer (NK) cells, but is not detected in a majority of resting T and B cells. We recently established a novel monoclonal antibody (mAb) to murine IL-2Rβ and examined in vivo the effect of the mAb in mice. We found that intraperitoneal injection of the anti-IL-2Rβ mAb into adult mice resulted in a selective in vivo elimination of splenic NK function in various mouse strains. The reduction of NK cell function is associated with complete disappearance of NK1.1$^+$ cells in C57BL/6 mice. Other lymphocyte subsets in the thymus and spleen were uncompromised. T cell function was not affected by the mAb treatment as judged by allogeneic cytotoxic T cell induction. The single injection of anti-IL-2Rβ mAb caused a long-term elimination of splenic NK cells, lasting for at least 5 wk. We also found that NK and/or NK precursor cells become susceptible to the mAb treatment only after birth, suggesting that functional maturation of NK cells in terms of IL-2Rβ expression is a later event in the course of NK cell development. The use of the anti-IL-2Rβ mAb will be useful in defining the physiological role of NK cells in host defense as well as dissecting their developmental pathway in vivo.

NK cells are large granular lymphocytes (LGL) that express MHC-nonrestricted cytotoxic activity. Several lines of evidence indicate that the NK cell is responsible for eradication of tumor cells (1, 2) and virally infected cells (3), however, roles of NK cells in immune defense remain to be fully elucidated.

IL-2 is a major growth factor for mature NK cells (4, 5). Freshly isolated NK cells preferentially express IL-2Rβ, through which IL-2 plays a pivotal role in proliferation and induction of cytolytic activity (6). IL-2Rβ is also expressed in other cell types, such as fetal T cells expressing Vγ5 TCR (7) and a subset of adult CD8$^+$ T cells (8). We recently reported that in utero treatment of mice with a neutralizing mAb against IL-2Rβ completely and selectively abrogates development of the Thy-1$^+$ dendritic epidermal cell (Thy-1$^+$ dEC), which is a descendant of the IL-2Rβ$^+$ Vγ5 TCR$^+$ cells (7).

In the present study, we report that postnatal treatment of mice with the anti-IL-2Rβ mAb (a single intraperitoneal injection) causes a long-lasting selective elimination of NK cells. We also report that NK cells and/or NK precursor cells become sensitive to anti-IL-2Rβ mAb treatment after birth.

Materials and Methods

Mice. Pregnant or male C57BL/6 and male C3H/He mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). Institute of Cancer Research (ICR) *nu/nu* mice and SCID (C.B17 *scid/scid*) mice were obtained from Charles River Japan Inc. (Atsugi, Japan) and Nihon Clea Inc. (Tokyo, Japan), respectively.

mAbs. The mAbs used were as follows: TM-β1 (anti-IL-2Rβ [8]), PK136 (anti-NK1.1 [9]), 145-2C11 (anti-CD3 [10]), H57-597 (anti-α/β TCR [11]), GL3 (anti-γ/δ TCR [12]), RM4-5 (anti-CD4 [Pharmingen, San Diego, CA]), 53-6.7 (anti-CD8 [13]), RA-3B2 (anti-B220 [14]), and M1/70 (anti-Mac-1 [15]).

Antibody Treatment. Adult mice were treated intraperitoneally with 1 mg of anti-IL-2Rβ mAb TM-β1 (rat IgG2b) or control normal rat IgG (Sigma Chemical Co., St. Louis, MO) in 500 μl of PBS as indicated. In a separate series of experiments, antibody injection was performed by two different protocols as follows. For in utero treatment, pregnant C57BL/6 mice were given intraperitoneal injection of 1 mg of anti-IL-2Rβ mAb daily from day 12 of gestation until birth as previously described (7). For postnatal treatment, neonatal mice were given a subcutaneous injection of ~50 μg of anti-IL-2Rβ mAb three times a week, which was continued until analysis. Injection volume was ~50 μl/d. Normal rat IgG was injected as a negative control in some experiments.

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Cell-mediated Cytotoxicity. Cytotoxic activity of NK cells was measured in a standard 4-h ^{51}Cr release assay (16). Briefly, 5 × 10^3 ^{51}Cr-labeled YAC-1 cells were mixed with a varied number of spleen cells and incubated for 4 h in 200 μl of the culture medium (RPMI 1640 [ICN Biomedicals Inc., Costa Mesa, CA], 10% FCS [Cell Culture Laboratories, Cleveland, OH], 10 mM Hepes, 2 mM L-glutamine, 50 μM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin). Nylon wool–nonadherent spleen cells (17) were used for a NK source in C57BL/6 mice. After incubation, the radioactivity in 100 μl of supernatant was measured and percent cytotoxicity was determined. Primary CTL were induced by culture of C57BL/6 mouse spleen cells (5 × 10^6) with mitomycin C–treated BALB/C mouse spleen cells (2.5 × 10^6) for 4 d in 2 ml of the culture medium. The cytotoxicity was determined as above by using P815 (H-2^d), RL-male1 (H-2^d), or EL-4 (H-2^b) cells as targets.

Flow Cytometry. Immunofluorescence and flow cytometric analysis was performed as described (8). At least 10,000 cells per sample were analyzed on EPICS-CS or EPICS-Elite flow cytometers (Coulter Electronics, Hialeah, FL).

Results and Discussion

Selective Long-Term Elimination of NK Cells by an Anti-IL-2Rβ mAb In Vivo. To evaluate the functional importance of IL-2Rβ expression in NK cells in vivo, adult mice were given an intraperitoneal injection of a function-blocking anti-IL-2Rβ mAb TM-β1 (8). NK activity in the spleen of antibody-treated mice was determined by using YAC-1 cells as a target. We found that an intraperitoneal injection with anti-IL-2Rβ mAb abrogated cytolytic activity of NK cells in various mouse strains, including C3H/He, C57BL/6, ICR *nu/nu*, and SCID mice (Fig. 1). As shown in Fig. 1, *C* and *D*, a single intraperitoneal injection of 1 mg of anti-IL-2Rβ mAb is sufficient for a profound reduction of NK activity in vivo.

Flow cytometric analysis was performed to determine whether the reduction of NK activity was caused by functional inactivation or elimination of NK cells by using C57BL/6 mice. As shown in Fig. 2, NK cells marked by expression of NK1.1 alloantigen were almost completely depleted in anti-IL-2Rβ-treated mice (Fig. 2 *B*), whereas they were readily detected in control IgG-treated mice (Fig. 2 *A*). Cells with lower levels of IL-2Rβ expression as compared with NK cells were still present after anti-IL-2Rβ mAb treatment (Fig. 2 *B*). This cell population most likely represents $CD8^+$ T cells, since it lacks NK1.1 antigen expression. Asialo-$GM1^+$ IL-$2R\beta^+$ cells were also depleted in anti-IL-2Rβ mAb–treated SCID mice (data not shown).

Further investigation revealed that the depletion was NK cell specific. First, similar numbers of cells were recovered from the spleen of anti-IL-2Rβ mAb- or control IgG-treated mice, and various splenic lymphocytes subsets remained intact in the mice treated with anti-IL-2Rβ mAb (Table 1). Second, lymphocyte composition in the thymus and lymph nodes were unaffected, and intraepithelial T cells, such as Thy-1^+ dEC and intestinal intraepithelial T cells, were also unchanged by this anti-IL-2Rβ mAb treatment (data not shown). Third, as shown in Fig. 3, alloantigen-reactive CTL

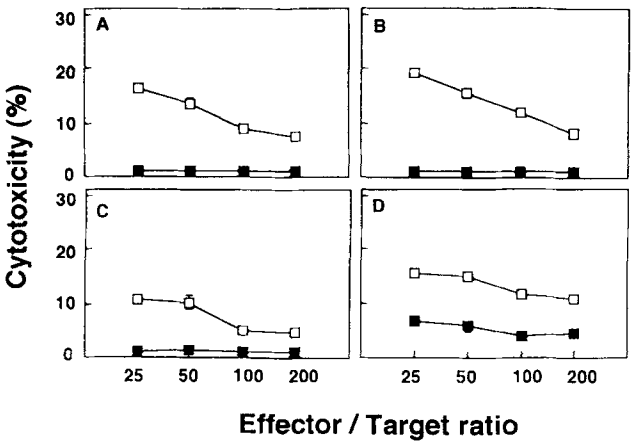

Figure 1. Treatment with anti-IL-2Rβ mAb abrogates splenic NK cell activity in adult mice. Mice were injected with 1 mg of anti-IL-2Rβ mAb TM-β1 (■) or normal rat IgG (□) in each treatment. Antibody treatment was performed three times every other day (*A* and *B*) or only once (*C* and *D*). NK activity was determined 2 d after the termination of antibody treatment by using YAC-1 cells as a target. (*A*) C3H/He, (*B*) ICR *nu/nu*, (*C*) C57BL/6, and (*D*) SCID.

were induced normally from the spleen of anti-IL-2Rβ mAb-treated mice.

Next we performed flow cytometric analysis at various time points after the single mAb treatment to determine the duration of the NK cell depletion. As shown in Fig. 4, $NK1.1^+$ IL-$2R\beta^+$ NK cells were hardly detectable in the spleen of the anti-IL-2Rβ-treated mice up to 5 wk after a single injection of the mAb, indicating that NK deficiency is long lasting. Ongoing experiment indicates that there is no sign of the recovery of $NK1.1^+$IL-$2R\beta^+$ cells in the spleen even 7 wk after the mAb injection (data not shown).

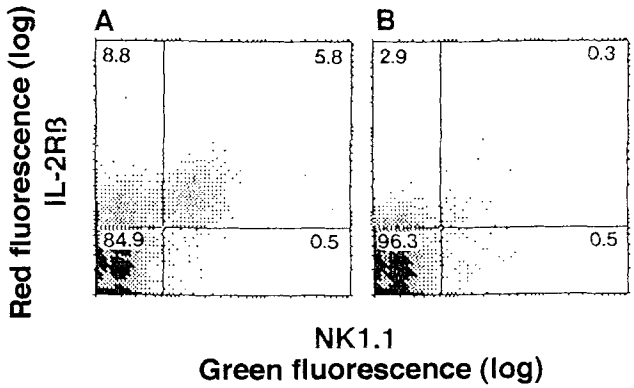

Figure 2. Anti-IL-2Rβ mAb treatment depletes splenic NK cells in vivo. C57BL/6 mice were given a single intraperitoneal injection of 1 mg of control normal rat IgG (*A*) or anti-IL-2Rβ mAb TM-β1 (*B*). Nylon wool–nonadherent spleen cells were incubated with a combination of FITC-conjugated PK136 (anti-NK1.1) and biotinylated TM-β1 (anti-IL-2Rβ) and stained with PE-conjugated streptavidin. Immunofluorescence was analyzed on an EPICS-CS flow cytometer (Coulter Electronics). The percentage of positively stained cells is indicated by the number in each quadrant.

Table 1. *Splenic Lymphocyte Subsets in Antibody-treated Mice*

	Positive cells (n = 3)	
	Control	Anti-IL-2Rβ
	% ± *SD*	
$CD3^+$	34.3 ± 3.3	34.3 ± 4.6
α/β TCR^+	33.7 ± 1.7	34.7 ± 3.7
γ/δ TCR^+	3.3 ± 0.5	4.0 ± 0.8
$CD4^+$	21.0 ± 0.8	22.3 ± 3.1
$CD8^+$	12.7 ± 0.9	12.7 ± 1.2
$B220^+$	51.7 ± 2.5	49.3 ± 1.2
$Mac\text{-}1^+$	3.3 ± 0.5	2.7 ± 0.9

C57BL/6 mice were treated intraperitoneally with 1 mg of anti-IL-2Rβ mAb (TM-β1) or control IgG. 2 d after antibody treatment, spleen cells from the mice (n = 3) were stained with FITC-conjugated mAb and examined for the presence of corresponding markers by an EPICS-CS flow cytometer. Mean values of percentages are shown ± SD.

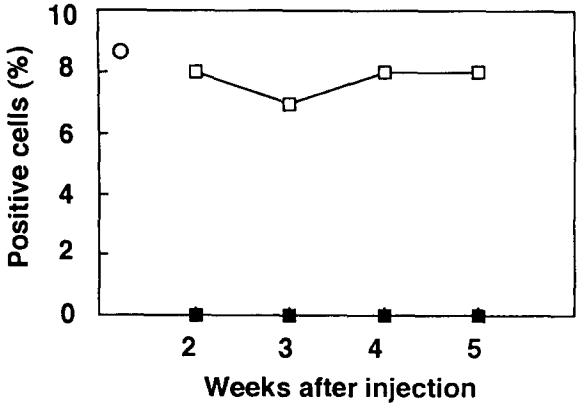

Figure 4. Long-term elimination of NK cells in vivo by a single injection of the anti-IL-2Rβ mAb. C57BL/6 mice were treated intraperitoneally with 1 mg of anti-IL-2Rβ mAb TM-β1 (■) or control normal rat IgG (□). $NK1.1^+IL\text{-}2R\beta^+$NK cells in the spleen from untreated (O) or antibody-treated mice were monitored at indicated time points after antibody treatment by flow cytometry as described in Fig. 2.

NK cell depletion in vivo was also successfully accomplished by administering antibody to cell surface antigens expressed in NK cells, such as asialo-GM1 (18), NK1.1 (19, 20), and 3.2.3 antigen (21). However, previous studies indicated that these antibodies caused only a short-term elimination of NK cells in vivo and that there was a substantial recovery of NK cells within 2 wk (18–21). Although the molecular mechanism(s) of disappearance of NK cells by anti-IL-2Rβ mAb treatment is not precisely known at present, the long-lasting effect of anti-IL-2Rβ mAb may suggest the possible involvement of the IL-2/IL-2R system in postnatal development of NK progenitor cells in vivo.

NK Cells Become Susceptible to Anti-IL-2Rβ mAb Treatment after Birth. A striking reduction of NK activity with ad-

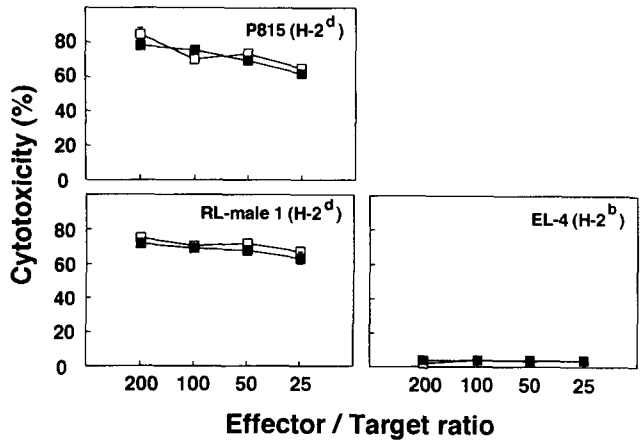

Figure 3. CTL induction from anti-IL-2Rβ mAb-treated mice. Primary CTL were induced by culture of spleen cells from the anti-IL-2Rβ mAb- (■) or control normal IgG- (□) treated C57BL/6 mice with mitomycin C-treated BALB/c mouse spleen cells for 4 d. The cytotoxicity was determined by using P815 ($H\text{-}2^d$), RL-male1 ($H\text{-}2^d$), or EL-4 ($H\text{-}2^b$) tumor cells as targets.

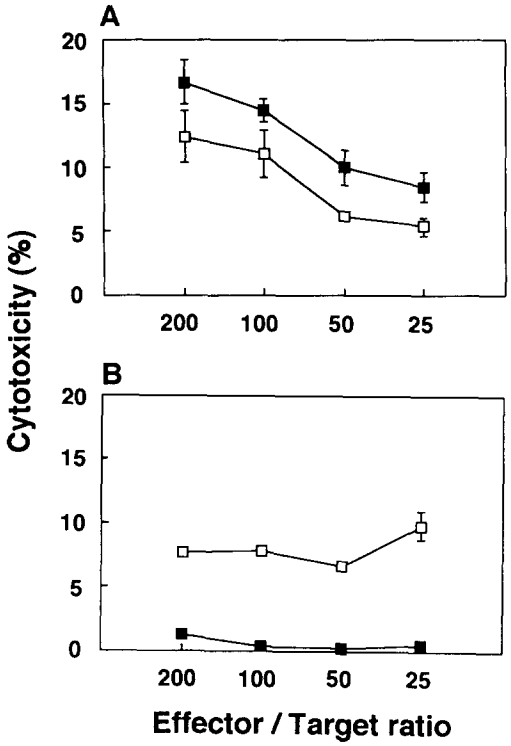

Figure 5. Postnatal but not in utero treatment with anti-IL-2Rβ mAb eliminates NK cell activity. In utero (*A*) or postnatal (*B*) treatment was performed as described in Materials and Methods. NK activity of nylon wool-nonadherent spleen cells from the C57BL/6 mice treated with (■) or without (□) anti-IL-2Rβ mAb TM-β1 was determined by using YAC-1 cells as a target at 6 wk of age.

ministration of anti-IL-2Rβ mAb prompted us to evaluate the effect of in utero or postnatal treatment with anti-IL-2Rβ mAb on NK cell development. In utero treatment was carried out by intraperitoneal injection of anti-IL-2Rβ mAb into pregnant C57BL/6 mice from day 12 of gestation until birth, and postnatal treatment was by subcutaneous injection of the mAb to neonatal mice three times a week, which was continued until analysis. Cytolytic activity of NK cells in antibody-treated mice was determined at 6 wk of age.

As shown in Fig. 5, the in utero treatment with anti-IL-2Rβ mAb did not reduce cytolytic activity of NK cells (Fig. 5 *A*), whereas the postnatal treatment with the same mAb completely abrogated cytotoxicity of NK cells against YAC-1 cells (Fig. 5 *B*). Flow cytometric analysis revealed that the in utero treatment did not alter development of NK cells in the spleen, whereas the postnatal treatment abolished the appearance of NK cells (data not shown). As previously described (7), the in utero treatment with the anti-IL-2Rβ mAb completely abrogated development of Thy-1$^+$ dEC, whose precursor is IL-2Rβ$^+$ fetal Vγ5TCR$^+$ cells, indicating that this treatment made saturating levels of anti-IL-2Rβ available in the fetal circulation during embryogenesis. These findings altogether suggest that NK cell and/or NK precursor cells become sensitive to anti-IL-2Rβ treatment after birth.

Differential susceptibility of fetal and postnatal NK cells to anti-IL-2Rβ mAb may suggest that induction or upregulation of IL-2Rβ expression in the NK cell is a later event in the course of NK cell development. In fact, our preliminary experiments showed that murine fetal liver of gestation day 14 contains a small proportion of NK1.1$^+$ cells that express considerably low levels of IL-2Rβ as compared with adult NK1.1$^+$ cells (T. Tanaka, unpublished observation). Recently, it was proposed that NK and T cells have a common origin (22–25). If NK and T cells do share the common pathway of development, the IL-2Rβ$^+$ cells found in fetal liver may be one of the candidates of the "NK cell-T cell progenitor." Further biochemical and genetic studies are needed to elucidate the biological relevance of this cell population in NK cell and T cell development.

In the present study, we demonstrated a selective and long-lasting elimination of NK cells by a single intraperitoneal injection of anti-IL-2Rβ mAb. The IL-2Rβ mAb may also help to generate animal models to which various types of human cells can be readily transplanted. Although SCID mice have been used for this purpose, it has been reported that a considerable proportion of human tumors actually fail to grow in SCID mice (26) possibly due to the NK activity observed in these mice (27, 28). We have observed that pretreatment of SCID mice with anti-IL-2Rβ mAb indeed favored the growth of freshly isolated adult T cell leukemia cells in vivo (29). Therefore, we believe that anti-IL-2Rβ mAb will afford the means of effectively manipulating NK cells in vivo.

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