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Immunology

HLA class I-specific inhibitory receptors in human T lymphocytes: Interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8$^+$ T cells

(T cell subsets)

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ABSTRACT **A fraction of human T lymphocytes, predominantly CD8$^+$, express receptors for HLA class I molecules typical of natural killer cells (natural killer receptors or NKRs) that inhibit T cell receptor-mediated functions. Herein, we analyzed possible mechanism(s) leading to the expression of NKRs by T cells responding to superantigens or allogeneic cells *in vitro*. We show that, in the presence of interleukin 15 (IL-15), T cells (depleted of NKR$^+$ cells) responding to toxic shock syndrome toxin 1 *de novo* express CD94, a molecule that is part of a heterodimeric NKR with a broad specificity for different HLA class I alleles. Maximal CD94 expression occurred when IL-15 was added shortly after the cells were placed into culture, and CD94 expression started 4–6 days after addition of IL-15. Although both CD4$^+$ and CD8$^+$ cells expressed CD94, the simultaneous expression of NKG2A (i.e., the other component of the CD94/NKG2A inhibitory NKR) was confined to CD8$^+$ cells. Similar data were obtained in T cell populations activated in mixed lymphocyte cultures in the presence of IL-15. The expression of CD94/NKG2A led to an impairment of allo-specific cytolytic activity by mixed lymphocyte culture-derived T cell populations or clones. Remarkably, cytolysis could be restored by the addition of anti-CD94 mAb, i.e., by masking the inhibitory NKRs.**

Natural killer (NK) cells have been shown to express inhibitory receptors specific for major histocompatibility complex (MHC) class I antigens (natural killer receptors or NKRs) (1, 2). The NKR–MHC class I interaction leads to inhibition of NK-mediated lysis of MHC class I$^+$ target cells (1–3). In mouse, the NKRs identified so far are represented by Ly49 molecules that are type II membrane proteins characterized by a C-type lectin domain (2). In humans, the HLA class I-specific inhibitory NKRs belong to two distinct molecular families. One is represented by members of the Ig superfamily and contains NKRs, characterized by two or three Ig-like domains that display specificity for different groups of HLA-C (p58.1 and p58.2 NKR), HLA-B (p70) (3–6), or HLA-A (p140) (7) alleles. The other NKR is a complex composed of the type II membrane proteins CD94 and NKG2A (8–12). CD94/NKG2A functions as a broad-specificity receptor recognizing molecules encoded by different HLA class I loci (9–11). Importantly, recent data have indicated that a minor subset of T lymphocytes that in normal donors is primarily represented by chronically activated cytolytic T lymphocytes express one or more NKRs (13–16). Also in T cells, NKRs either belong to the Ig superfamily or are represented by CD94/NKG2A (14). NKR engagement leads to inhibition of T cell functions including T cell receptor (TCR)-mediated triggering of cytolytic activity and lymphokine production (13–17). The expression of inhibitory NKRs that counteract the function of cytotoxic T lymphocytes (CTLs) is potentially harmful to the host, as suggested by recent data from HIV infections (18) or from patients with melanoma (19). Therefore, it is important to define the mechanisms leading to the expression of inhibitory NKR by T lymphocytes. In this context, we showed that CD7$^+$ CD34$^+$ immature cell precursors can undergo maturation toward NK cells when cultured in the presence of interleukin 15 (IL-15) and the resulting NK cells selectively expressed CD94/NKG2A as the only HLA class I-specific inhibitory receptor (20, 21).

The present study was based on the hypothesis that IL-15 could induce expression of NKRs in mature T cells upon activation. Indeed, we show that, in the presence of IL-15, T cells activated by superantigens or allogeneic cells may *de novo* express the CD94/NKG2A receptor molecules but not NKRs belonging to the Ig superfamily. Surface expression of CD94/NKG2A was essentially confined to CD8$^+$ cells. Importantly, the specific lysis of allogeneic cells by allo-specific CTL populations or clones that expressed CD94/NKG2A could be restored by antibody-mediated masking of CD94 molecules.

MATERIALS AND METHODS

Antibodies and Reagents. mAbs XA185 (IgG1, anti-CD94), Y9 (IgM, anti-CD94), GL183 (IgG1, anti-p58.2), EB6 (IgG1, anti-p58.1), KD1 (IgG2a, anti-CD16), HP26 (IgG2A, anti-CD4), B9.4 (IgG2b, anti-CD8), Q66 (IgM, anti-p140), JT3a (IgG2a, anti-CD3), JTi4 (IgG2b, anti-Vβ8), Z270 (IgG1, anti-NKG2-A), A6136 (IgM, anti-HLA class I), 6A4 [F(ab′)$_2$, anti-class I; selected in our laboratory, see refs. 3, 7, and 20], and anti-Vβ2 (IgM, provided by F. Romagné, Immunotech, Marseille) were used in this study.

Fluorescein isothiocyanate- and phycoerythrin-conjugated anti-isotype goat anti-mouse antibodies were purchased from Southern Biotechnology (Birmingham, AL). The culture medium used was RPMI medium 1640 (Seromed, Berlin) supplemented with 10% fetal calf serum (Boehringer), 1% antibiotic mixture [penicillin (5 mg/ml)/streptomycin (5 mg/ml)/

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Abbreviations: NK, natural killer; NKR, NK receptor; TCR, T cell receptor; IL, interleukin; TSST-1, toxic shock syndrome toxin 1; MLC, mixed lymphocyte culture; CTL, cytotoxic T lymphocyte; PBL, peripheral blood lymphocyte; PHA, phytohemagglutinin.

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neomycin stock solution (10 mg/ml)] and human recombinant IL-2 was provided by Cetus (Emeryville, CA).

Human recombinant IL-15, human recombinant IL-7, and human recombinant IL-6 were purchased from PeproTech (Boston). Human recombinant IL-1 was purchased from Genzyme. Human recombinant IL-12 was purchased from R&D Systems. Phytohemagglutinin (PHA) was purchased from GIBCO/BRL. Toxic shock syndrome toxin 1 (TSST-1) or staphylococcal enterotoxin E superantigens were purchased from Toxin Technology (Madison, WI). Ficoll/Hypaque (Histopaque 1077) was purchased from Sigma.

Immunofluorescence Analysis. The reactivity of mAbs with cell populations was assessed by indirect immunofluorescence and flow cytometry analyses, as described (22). Briefly, 10^5 cells were stained with the corresponding mAb followed by a fluorescein isothiocyanate-conjugated goat anti-mouse Ig or appropriate fluorescein isothiocyanate- or phycoerythrin-conjugated anti-isotype-specific goat anti-mouse antiserum (Southern Biotechnology) as second-step reagents. All samples were analyzed on a flow cytometer (FACSort, Becton Dickinson). Results are expressed as logarithm of green fluorescence intensity (arbitrary units) versus logarithm of red fluorescence intensity.

T Lymphocytes and Cell Cultures. Peripheral blood lymphocytes (PBLs) were isolated from blood of normal donors by a Ficoll/Hypaque density gradient, as described (16).

Cells were incubated in the presence of TSST-1 (5 ng/ml) or staphylococcal enterotoxin E (5 ng/ml) superantigens, as described (22). In some experiments, PBLs were depleted of $CD94^+$, $CD4^+$, or $CD8^+$ cells by negative selection using respectively, XA185, HP26, or B9.4 mAbs and magnetic beads coated with anti-mouse Ig (Immunotech, Luminy, France). The following cytokines were added to cultures as indicated (days 2, 4, or 6): IL-2, 5–200 units/ml; IL-15, 1–10 ng/ml; IL-7, 1–10 ng/ml; IL-1, 5–200 units/ml; IL-12, 0.1–2 ng/ml; IL-6, 1–10 ng/ml.

Mixed lymphocytes cultures (MLCs) were set up as described (23) by culturing PBLs in round-bottom microwells (10^5 cells per well) with irradiated (5,000 rads; 1 rad = 0.01 Gy) allogeneic PBLs (2×10^5 cells per well). Two days after the cells were placed into culture, various cytokines were added at various times (on day 2, 4, and 6 of culture).

Cell Cloning. Clones were derived under limiting dilution conditions (16, 22). Lymphocyte suspensions were derived from TSST-1-stimulated cells cultured for 2 days or from MLCs cultured for 5 days. Microcultures were supplemented with either IL-15 or IL-2 and were cultured for 3–5 weeks before phenotypic and functional analysis. In some experiments, IL-15 and/or IL-2 was added to CD94/NKG2A-negative T cell clones.

Assay for Cytolytic Activity. The cytolytic activity of MLC populations, obtained either in the presence or in the absence of IL-15 or IL-2, was tested against ^{51}Cr-labeled allogeneic PHA blasts bearing the stimulating alloantigens, in the presence or in the absence of anti-CD94 mAb (Y9, IgM) or anti-class I [A6136, IgM, and 6A4, IgG $F(ab')_2$]. Data are expressed as percent of specific lysis at the indicated effector/target ratios.

RESULTS

***De Novo* Expression of CD94 in TSST-1-Stimulated Peripheral T Lymphocytes in the Presence of IL-15.** In a first series of experiments, we analyzed whether IL-15 could induce the expression of CD94 molecules in peripheral blood T lymphocytes cultured in the presence of the TSST-1 superantigen. To this end, because CD94 is present on a small fraction of fresh T lymphocytes (as well as on NK cells), $CD94^+$ cells were removed. The resulting $CD94^-$ mononuclear cells were used as responding populations. Cells were stimulated with TSST-1 in the presence or in the absence of IL-15. Other cytokines used included IL-1, IL-2, IL-6, IL-7, or IL-12. Cytokines were added on day 2 after the initiation of culture and the expression of CD94 was evaluated at day 10. $CD94^+$ cells were detected in cultures containing IL-15, whereas lower percentages were found also in cultures containing IL-2 (see below). Virtually no $CD94^+$ cells could be detected in control cultures (i.e., with no added cytokine, see below) or in cultures containing various concentrations of the interleukins indicated above (data not shown). Because expression of CD94 was detected only in cultures supplemented with IL-15 and IL-2 (which share both the β and γ chains of their receptors) (24, 25), we analyzed various concentrations for both cytokines. For both cytokines, the maximal percent of $CD94^+$ cells was obtained with IL-15 at 5 ng/ml and with IL-2 at 10 units/ml (data not shown). These cytokine concentrations were used in the subsequent experiments. Double fluorescence analysis confirmed that all the $CD94^+$ cells detected in the culture combinations supplemented with IL-15 were $CD3^+$ (Fig. 1*A*). In addition, the majority of $CD94^+$ cells were stained by a mAb specific for the β chain variable region Vβ2 segment of the TCR (specifically binding TSST-1, with consequent proliferation of $V\beta2^+$ T cells; Fig. 1*B*). Similar results were obtained with staphylococcal enterotoxin E, another bacterial superantigen (data not shown). To document the time course for the induction of

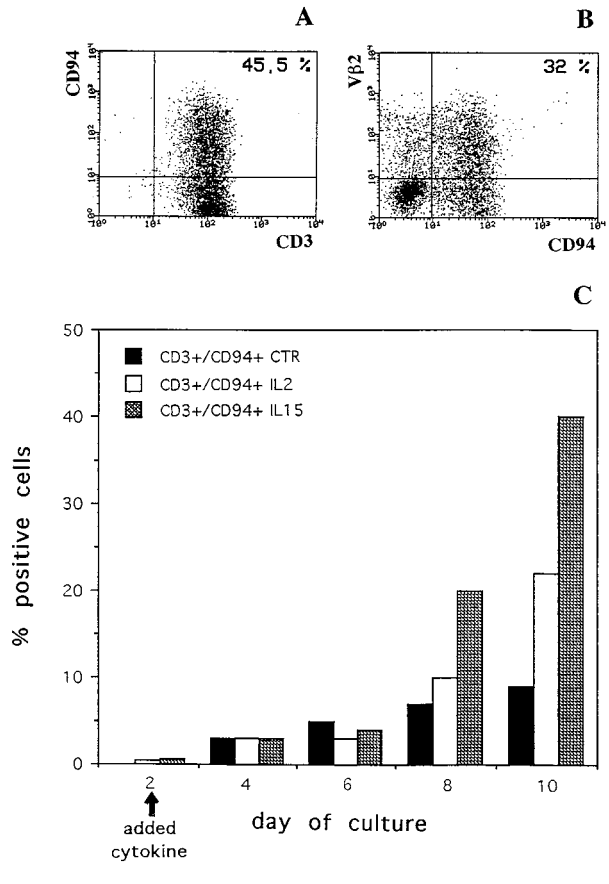

FIG. 1. IL-15-induced surface expression of CD94 molecules in TSST-1-stimulated T lymphocytes. PBLs depleted of $CD94^+$ cells were stimulated with TSST-1 (specific for the Vβ2 segment of TCR). IL-15, IL-2, or culture medium was added at 2 days after the initiation of culture. Cells derived from day 10 cultures supplemented with IL-15 were analyzed by double fluorescence for the expression of CD94 and CD3 (*A*) or CD94 and Vβ2 (*B*). *x* axis, green fluorescence; *y* axis, red fluorescence. (*C*) Time course of CD94 expression. In this experiments, IL-15, IL-2, or culture medium was added at day 2. Cultures were continued up to day 10, and the proportion of $CD3^+$ cells coexpressing CD94 was evaluated by flow cytometry in cultures supplemented with IL-15, IL-2, or medium, as indicated.

CD94 expression, IL-15, IL-2, or culture medium was added on day 2 and cultures were continued up to day 10. The proportion of cultured CD94$^+$ CD3$^+$ cells was evaluated at various times. At the time of cytokine addition, cultures did not contain CD94$^+$ CD3$^+$ cells. As shown in Fig. 1*C*, a substantial increase occurred at days 8–10 in the presence of IL-15 and, to a lower extent, in the presence of IL-2.

We next analyzed whether the expression of CD94 required an early addition of IL-15 after initiation of the cultures. IL-15 was added at days 2, 4, and 6 of culture, and the proportions of CD3$^+$ cells expressing CD94 were evaluated at days 10 and 12. Maximal expression was consistently detected (in three experiments) upon addition of IL-15 at day 2 (data not shown). This was true also in cultures supplemented with IL-2, although they contained lower proportions of CD94$^+$ cells. That an early addition of IL-15 was required to induce expression of CD94 was also suggested by the analysis of a series of CD94$^-$ (TSST-1-induced) T cell clones. The addition of IL-15 alone or in combination with IL-2 could not induce CD94 expression (data not shown). Although not shown, under the same experimental conditions, expression of NKRs belonging to the Ig superfamily, including p58.1, p58.2, p70, and p140, was not detected.

Expression of CD94 or NKG2A by the CD8$^+$ or CD4$^+$ Subsets in T Cells Stimulated with TSST-1 in the Presence of IL-15. Because CD94 may be expressed in association with NKG2A to form an HLA class I-specific inhibitory receptor, we further analyzed TSST-1-stimulated lymphocytes cultured in the presence of IL-15 for the expression of NKG2A. In addition, we investigated the pattern of expression of CD94 and NKG2A in CD4$^+$ or CD8$^+$ subsets. In this context, it is now well established that both CD4$^+$ and CD8$^+$ cells expressing the appropriate TCR Vβ can be activated by bacterial superantigen, in the presence of HLA class II$^+$ cells (26). A difficulty in planning these experiments was the preferential proliferation of CD4$^+$ when unfractionated T cells were activated by TSST-1 (data not shown), whereas expression of NKRs appeared to be primarily associated with CD8$^+$ cells (14, 16). In agreement with previous data, Fig. 2 *A*, *B*, *E*, and *F* shows that, in fresh unfractionated PBLs, CD94$^+$ and NKG2A$^+$ cells are confined to the CD8$^+$ subset. To assess whether CD8$^+$ cells could express CD94/NKG2A in TSST-1-induced cultures, lymphocytes to be tested were enriched in CD8$^+$ (or CD4$^+$) cells. To this end, PBLs were depleted of CD4$^+$ (or CD8$^+$) cells and cultured in the presence of TSST-1. IL-15 was added at day 2. As shown in Fig. 2 *C* and *G*, both populations enriched in CD4$^+$ or CD8$^+$ cells expressed CD94. In contrast, expression of NKG2A was mostly confined to CD8$^+$ cells (Fig. 2 *D* and *H*). This further supports the concept that in T cells, the inhibitory CD94/NKG2A receptor complex is primarily expressed by CD8$^+$ cells.

Expression of CD94 or NKG2A in T Lymphocytes Activated in MLCs in the Presence of IL-15. The following studies were designed to analyze whether T lymphocytes responding to specific antigens in the presence of IL-15 could express CD94 and NKG2A molecules and, if so, whether the expression of CD94/NKG2A NKRs could interfere with TCR-mediated antigen-specific functions. The model of T cell stimulation in MLC was selected because, in this assay, both alloreactive CD4$^+$ and CD8$^+$ cells undergo proliferation. Moreover, the resulting alloantigen-specific CD8$^+$ CTLs can be analyzed in specific cytolytic assays.

MLCs were established by using unfractioned PBLs as responder cell populations. IL-15 or IL-2 were added 2 days after the initiation of culture. Controls were MLCs to which no exogenous cytokine had been added. Fig. 3 shows a representative experiment. On day 0, the proportions of fresh CD3$^+$ cells expressing either CD94 or NKG2A are shown. Fig. 3 also shows MLC-derived cell populations that have been cultured for 8 days under the indicated conditions. It is evident that MLC populations supplemented with IL-15 contained relatively high proportions of CD94$^+$ cells, which were primarily confined to CD8$^+$ cells. The pattern of expression of NKG2A was similar to that of CD94 in both CD3$^+$ and in CD8$^+$ populations (although the percentages of NKG2A$^+$ cells were consistently lower than those of CD94$^+$ cells). In CD4$^+$ cells, the percentages of CD94$^+$ and NKG2A$^+$ were low. Similar results were obtained in five other experiments using different responder/stimulator combinations. Therefore, expression of the CD94/NKG2A inhibitory NKR is confined to CD8$^+$ cells in this *in vitro* system also. In addition, not all CD8$^+$ cells that expressed CD94 were also NKG2A$^+$. Control cultures on day 8 or cultures supplemented with IL-2 contained, in the various T cell populations analyzed, lower proportions of CD94$^+$ and

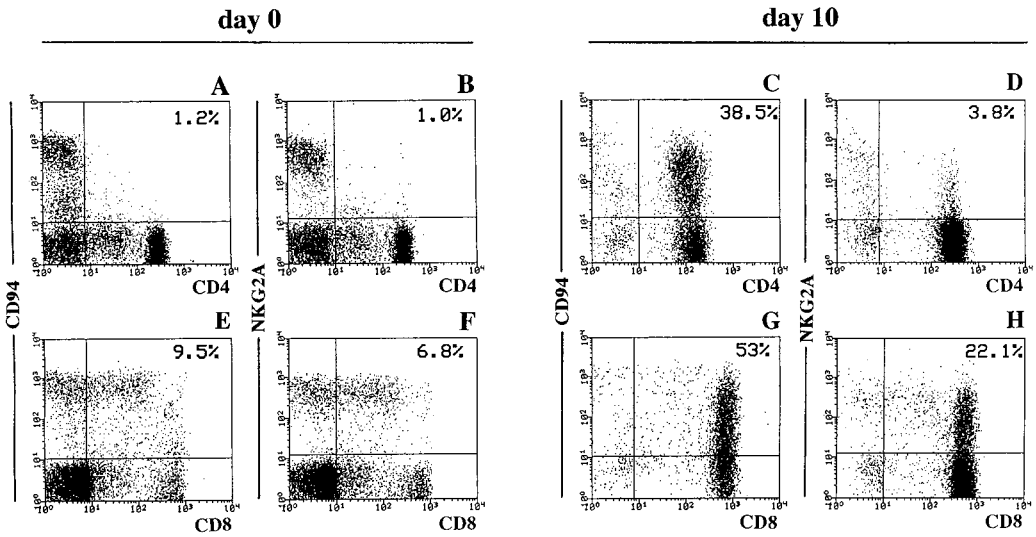

FIG. 2. IL-15-induced expression of CD94 or NKG2A in TSST-1-stimulated lymphocyte populations enriched in CD4$^+$ or CD8$^+$ cells. (*A*, *B*, *E*, and *F*) Fresh unfractionated peripheral blood lymphocytes were analyzed for the coexpression of CD94 or NKG2A and CD4 or CD8 antigens, respectively. To assess the expression of CD94 or NKG2A in TSST-1-stimulated CD4$^+$ or CD8$^+$ subsets in the presence of IL-15, lymphocytes were depleted of CD4$^+$ or CD8$^+$ cells prior to culture with TSST-1 and addition of IL-15 at day 2. The expression of CD94 or NKG2A by CD4$^+$ (*C* and *D*) or CD8$^+$ (*G* and *H*) cells cultured for 10 days is shown. Cells have been analyzed by flow cytometry (*x* axis, green fluorescence; *y* axis, red fluorescence).

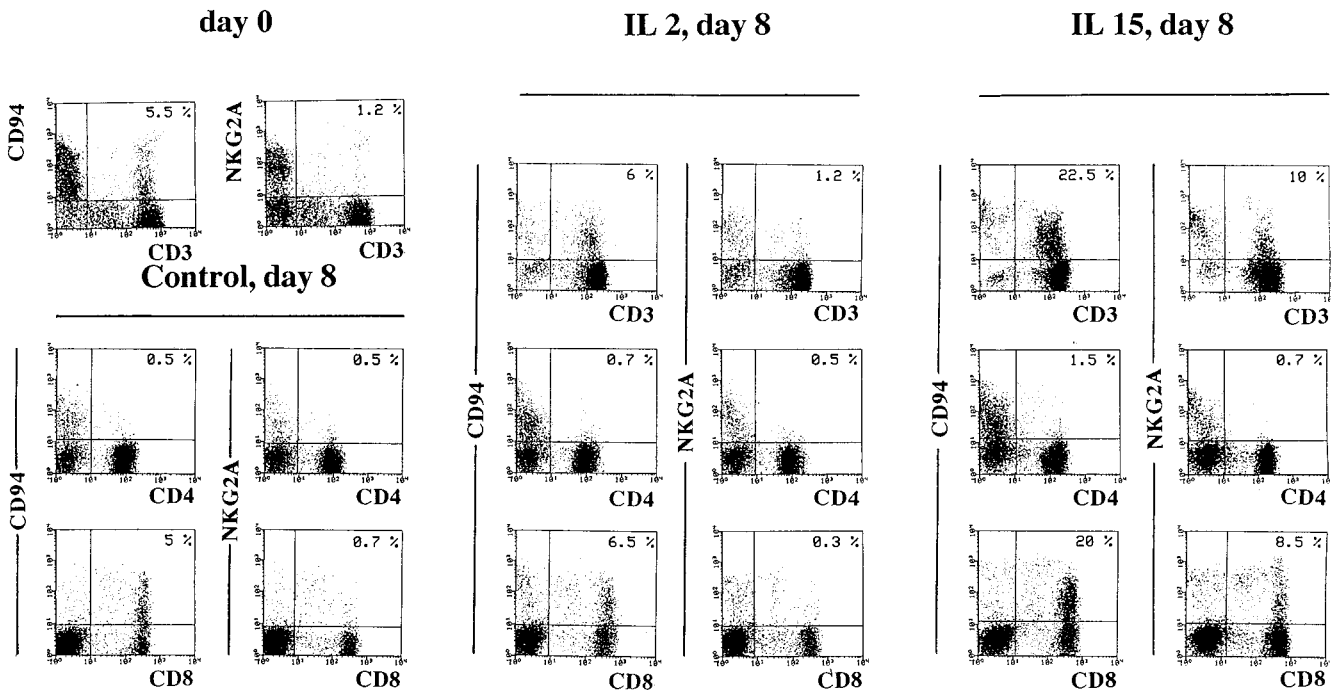

FIG. 3. Effect of IL-15 or IL-2 on the expression of CD94 or NKG2A molecules in MLC-activated T cells. The expression of CD94 or NKG2A by $CD3^+$ cells in fresh PBLs that have been used as responder population in MLCs is shown at day 0. These lymphocytes were cultured with irradiated allogeneic cells. IL-15, IL-2, or culture medium (control) were added at day 2 and cultures were harvested at day 8. Cells cultured under the indicated conditions were stained for CD94 or NKG2A in combination with CD3, CD4, or CD8. Cells have been analyzed by flow cytometry (*x* axis, green fluorescence; *y* axis, red fluorescence).

$NKG2A^+$ cells. Therefore, IL-15 appears to be an efficient inducer of the expression of CD94 and NKG2A also in alloantigen-stimulated $CD8^+$ T lymphocytes.

CD94/NKG2A-Induced Inhibition of Specific Target Cell Lysis Mediated by Alloreactive CTL Populations or Clones. The above data indicate that a fraction of MLC-activated $CD8^+$ cells expressed CD94/NKG2A when cultured in the presence of IL-15. Because these cells are likely to include alloreactive CTLs, we further investigated whether CD94/NKG2A receptor could mediate inhibition of the specific cytolysis of target cells bearing the stimulating alloantigens. When MLCs that had been supplemented with IL-15 were cultured for a longer time, at least in some donors, the proportions of $CD94^+$ and $NKG2A^+$ cells further increased. For example, in the experiment shown in Fig. 4*A*, a large fraction of day 12 MLC T cells expressed CD94 and approximately one-third of these also expressed NKG2A (Fig. 4*B*). Moreover, in this donor, the large majority of MLC cells were $CD8^+$ (Fig. 4*C*). The specific CTL activity of these cells was analyzed in comparison with that of day 12 MLC cells cultured in IL-2. Remarkably, the latter population contained only 5% of $CD94^+$ cells and $<1\%$ $NKG2A^+$ cells (data not shown). A representative experiment is shown in Fig. 4*D*. The cytolytic activity of cells cultured in IL-15 was significantly lower than that of cells cultured in IL-2. Therefore, we analyzed whether this was consequent to the interaction of CD94/NKG2A with the HLA-class I molecules expressed on target cells. To this end, the cytolytic test was performed also in the presence of anti-CD94 mAb. mAb-mediated masking of CD94/NKG2A resulted in a substantial increase in cytolytic activity (Fig. 4*D*). No such effect could be detected in MLC populations cultured in the presence of IL-2. Similar data were obtained in four experiments using different responder/stimulator combinations in which the MLC populations were cultured in IL-15 or IL-2 for 11–15 days. The addition of anti-HLA-class I mAb, used as control, strongly inhibited the cytolytic activity of both MLC populations. Note that, in the experiment shown, the cell population analyzed was mostly $CD8^+$ (Fig. 4*C*) and, thus, only included HLA class I-restricted CTLs. In addition, note that the frequency of alloreactive $CD4^+$ cytolytic cells isolated from MLC is rather low, as demonstrated by limiting dilution experiments (23).

To further analyze the interference of CD94/NKG2A with the specific cytolytic activity of $CD8^+$ alloreactive CTLs, we studied a series of MLC-derived CTL clones. Clones were obtained under limiting dilution conditions from MLC populations supplemented with IL-15. Numerous $CD8^+$ clones displaying specific cytolytic activity were found to express CD94/NKG2A (data not shown). As expected the specific cytolytic activity of $CD8^+$ T cell clones expressing CD94/NKG2A was generally lower than that of $CD8^+$ $CD94^+/NKG2A^-$ clones. The representative $CD94^+/NKG2A^+$ clones KK20 and KK26 killed allogeneic cells with lower efficiency than $CD94^+/NKG2A^-$ clones. However, in the presence of anti-CD94 mAb, their cytolytic activity was similar to that of $CD8^+$, $CD94^+/NKG2A^-$ clones (Fig. 4*E*). Note that although clone KK11 ($CD94^+/NKG2A^+$) lysed target cells with an efficiency similar to that of $CD94^+/NKG2A^-$ clones (KK18 and KK27), its activity was considerably enhanced in the presence of anti-CD94 mAb (Fig. 4*E*). Thus, anti-CD94 mAb consistently increased the percent of specific lysis in $CD94^+/NKG2A^+$ clones but not in $CD94^+/NKG2A^-$ clones.

DISCUSSION

The present study provides evidence that T lymphocytes activated in the presence of IL-15 *de novo* express the HLA class I-specific inhibitory receptor CD94/NKG2A. This occurs in $CD8^+$ cells whereas $CD4^+$ cells may express CD94 but not NKG2A. Perhaps more importantly, expression of CD94/NKG2A resulted in down-regulation of TCR-mediated functions as indicated by the impairment of specific allogeneic cell lysis by MLC-activated CTLs.

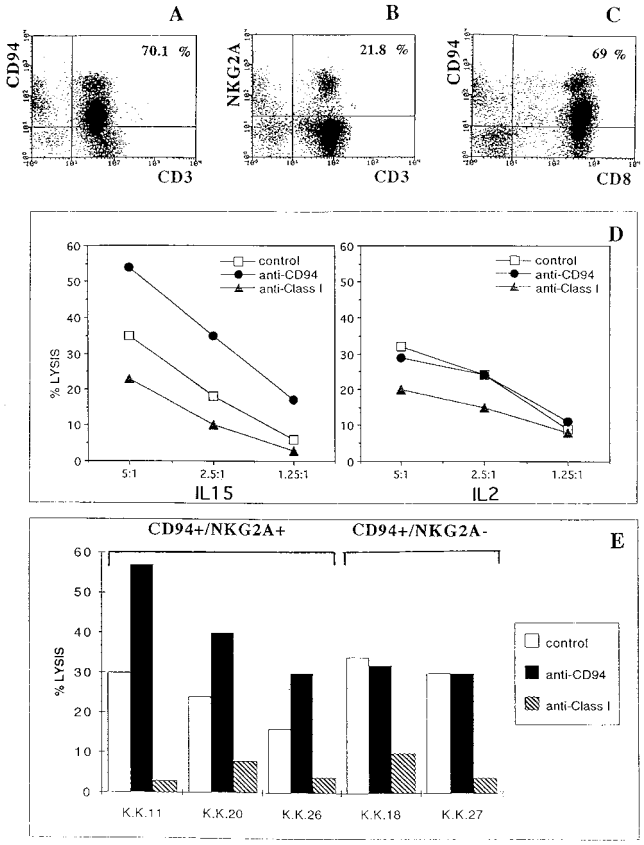

FIG. 4. CD94/NKG2A receptor inhibits the specific cytolytic activity of MLC-derived T cell populations or clones. Lymphocytes were cultured in MLCs with irradiated allogeneic cells. IL-15 or IL-2 were added at day 2 and cultures were continued for additional 10 days. Cells cultured in IL-15 were harvested and analyzed for the coexpression of CD94 or NKG2A and CD3 (*A* and *B*). (*C*) Most cells recovered in this experiment were $CD8^+$. Parallel cultures supplemented with IL-2 contained only 5% $CD94^+$ T cells. $NKG2A^+$ cells were <1% (data not shown). Both MLC populations were analyzed for cytolytic activity (*D*) against ^{51}Cr-labeled PHA blasts bearing the stimulating alloantigens at different effector/target ratios either in the absence of added mAb (□) or in the presence of anti-CD94 (●) or anti-HLA-class I mAb (▲). Each point represents the mean of duplicate experiments. (*E*) Three representative allospecific $CD8^+$ clones expressing both CD94 and NKG2A (KK11, KK20, and KK26) are shown. Two allospecific $CD8^+$ $CD94^+$ clones that did not express NKG2A are shown for comparison. Again, the effect of addition of anti-CD94 mAb (solid bars) on the specific lysis of ^{51}Cr-labeled PHA blasts derived from the donor used as stimulator is evaluated in comparison to controls (i.e., no mAb added, open bars) or to cytolytic assays to which anti-class I mAb has been added (hatched bars). The effector/target ratio was 2:1.

As shown (21), in the presence of IL-15, $CD34^+$ $CD7^+$ precursors isolated from human thymus undergo maturation toward NK cells and express CD94/NKG2A NKRs. Therefore, it appears that IL-15 may play a more general role because it can induce the *de novo* expression of CD94/NKG2A also in mature T cells undergoing activation. The finding that also IL-2 may induce the expression of CD94, although to a lower extent, is likely to reflect the fact that both IL-15 and IL-2 receptor complexes in T and NK cells share the β and γ chains (24, 25). This may explain why IL-2 and IL-15 share a number of biological activities, including the induction of cell proliferation, cytolytic activity, and lymphokine production in T and NK cells (24). On the other hand, the two receptor complexes have different α chains. Differences in the ligand-binding affinity of the α chains of the receptors for IL-15 and IL-2 (25) might explain the differential ability of IL-15 and IL-2 to induce CD94/NKG2A surface expression. Remarkably, although a second type of IL-15 receptor has been identified (termed IL-15RX), this receptor has been identified in mast cells but not in T or NK cells (27). CD94/NKG2A has been shown to function as a broad-specificity receptor recognizing the products of different HLA class I loci (9, 11). Thus, when expressed by CTLs, it can interact with the various HLA class I alleles expressed on target cells and inhibit the TCR-mediated cytolysis. This was directly demonstrated in the present study. Thus, the specific CTL activity of $CD8^+$/$CD94^+$/$NKG2A^+$ MLC-induced T cell populations or T cell clones was substantially increased upon mAb-mediated blocking of CD94/NKG2A. These data support the concept that CTLs expressing inhibitory NKRs are not anergic but are simply inhibited in their function as a consequence of the interaction between NKR and HLA class I molecules.

Activated $CD4^+$ cells were found to express CD94 but not NKG2A. In NK cells lacking NKG2A, CD94 may be associated with other members of the NKG2 family (such as the NKG2C) or with still undefined molecules (12, 28). Engagement of CD94 molecules in these NK clones led either to cell triggering or to no functional effect. It should be noted, however, that no specificity for conventional HLA class I molecules has been demonstrated for the activating form of CD94 receptor complex. Preliminary analysis of $CD4^+$ $CD94^+$ cells did not allow us to demonstrate either cell triggering or inhibition of cell function. Although in the culture systems analyzed (and in fresh lymphocytes from normal donors) virtually no $CD4^+$ cells expressing CD94/NKG2A could be detected, we cannot exclude that it may be expressed also by $CD4^+$ cells at least under certain conditions. For example, it will be interesting to investigate whether "polarized" forms of $CD4^+$ cells such as Th1 and Th2 cells (29) may express CD94/NKG2A.

Peripheral T cells from normal donors express not only CD94/NKG2A but also NKR belonging to the Ig superfamily, including p58.1, p58.2, p70, and p140 (3, 5–7, 30). IL-15 failed to induce the expression of these receptors both in $CD34^+$ $CD7^+$ precursors undergoing maturation toward NK cells (21) and in mature T cells. It is noteworthy that genes encoding both CD94 and NKG2A are located on the human chromosome 12 (in the NK gene complex) but that those belonging to the Ig superfamily are on chromosome 19 (1, 5). It is possible that stimuli (cytokines?) required for inducing transcription of the second group of genes may be, at least in part, different. The finding that CD94 and NKG2A were expressed in the presence of IL-15 suggests that this cytokine may be sufficient to induce (directly or indirectly) the transcription of the genes encoding CD94 and NKG2A. IL-15 has been detected in activated macrophages, in fibroblasts, in some epithelial cells, and in placenta (24, 25). It is possible that CD94/NKG2A may be expressed *in vivo* when T cell activation occurs at particular sites, perhaps involving antigen-presenting cells or target cells that actively synthesize IL-15.

Understanding of the mechanism regulating the surface expression of CD94/NKG2A or other inhibitory NKRs may be particularly important for the implications in the negative regulation of T cell responses. The expression of inhibitory NKRs may be detrimental to the host in case of CTL responses to cytopathic viruses or tumor cells. In this context, in some HIV-infected patients, HIV-specific CTLs were found to express inhibitory NKRs (18). In addition, a melanoma-specific CTL clone has been isolated that expressed p58.2 (specific for the self HLA-Cw7 allele) and failed to lyse the autologous tumor cells (19). On the other hand, the expression of inhibitory NKRs by autoreactive T lymphocytes could be potentially useful in certain autoimmune diseases. Moreover, a similar favorable situation can be envisaged if NKR expression occurs *in vivo* in alloreactive CTLs during transplantation. Along this line, it has recently been shown that, in transgenic mice, the transgenic expression of the p58.2 NKR prevents the

rejection of transgenic H-2-mismatched bone marrow graft that expresses the HLA-Cw3 allele (recognized by p58.2) (31). These data, our present findings, and previous findings (21) emphasize the potential implication of cytokine-induced NKR expression in the development of novel strategies to prevent allograft rejection or graft versus host disease.

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