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T-cell-receptor-independent activation of cytolytic activity of cytotoxic T lymphocytes mediated through CD44 and gp90^{MEL-14}

(lymphocyte homing receptors/endothelial cells/adhesion molecules)

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ABSTRACT **CD44 is a transmembrane glycoprotein found on a variety of cells including those of myeloid and lymphoid origin. CD44 is highly conserved among various species and is involved in the homing of lymphocytes and monocytes to lymph nodes, Peyer's patches, and sites of inflammation. In the present study, we demonstrate that monoclonal antibody (mAb) 9F3, directed against murine phagocytic glycoprotein 1 (CD44) expressed on cytotoxic T lymphocytes (CTLs), can trigger the lytic activity of CTLs and redirect CTL-mediated lysis to antigen-negative Fc receptor-positive target cells. Similar redirected lysis was also inducible using mAb MEL-14, directed against the lymphocyte homing receptor for endothelium (gp90^{MEL-14}). The redirected lysis induced by mAbs 9F3 and MEL-14 was similar to that induced by mAbs against the $\alpha\beta$ T-cell receptor or CD3. In contrast, mAbs directed against CD8, CD45R, and CD11a (LFA-1, lymphocyte function-associated antigen 1) failed to evoke lytic activity. The current study demonstrates that CD44 and gp90^{MEL-14} molecules, in addition to participating in T-cell homing and adhesion, may play a major role in delivering the transmembrane signal to the CTL that triggers the lytic activity, even when the T-cell receptor is not occupied. Such a mechanism may account for the nonspecific tissue damage seen at sites of CTL-mediated inflammation.**

CD44 antigen is a transmembrane glycoprotein found on lymphoid and myeloid cells (for review, see ref. 1). CD44 has been found to be identical to a variety of other molecules such as phagocytic glycoprotein 1 (Pgp-1), expressed on macrophages and granulocytes; Ly-24 antigen, present on T-cell precursors; Hermes lymphocyte homing receptor, which plays a role in the binding of lymphocytes to the high endothelial venules; blood group antigen, In(Lu), expressed on erythrocytes and monocytes; fibroblast extracellular matrix receptor type III, which links the cytoskeleton with the extracellular matrix; and the Hutch-1 molecule, involved in lymphocyte-endothelial cell binding (for review, see ref. 1). CD44 has been shown to be highly conserved among various species (2), although its exact functions in various cell types remain unclear.

Although early reports suggested that CD44 was expressed only by immature or precursor T cells in the thymus, subsequent studies demonstrated that CD44 is also expressed by mature peripheral T cells (for review, see ref. 3). Furthermore, peripheral T cells have been shown to upregulate CD44 expression after activation by the CD3–T-cell receptor (TCR) complex, thereby suggesting that CD44 may represent a T-cell activation antigen (1). CD44 may also be involved in T-cell adhesion and activation, because anti-CD44 monoclonal antibodies (mAbs) enhance T-cell proliferation induced by anti-CD3 and anti-CD2 (4–6). Recent studies have also shown that anti-CD44 antibodies block lympho-hemopoiesis in long-term bone marrow cultures (7). MEL-14 is a mAb specific for the lymphocyte surface molecule gp90^{MEL-14}, which recognizes high endothelial venules and is involved in organ-specific homing of lymphocytes (8). mAb MEL-14 has been shown to recognize the human Hermes (CD44) molecule and to be functionally related to this antigen. However, cloning and sequencing of gp90^{MEL-14} and CD44 genes have suggested that structurally these molecules may not be related (1).

Cytotoxic T lymphocytes (CTLs) can be activated by antibodies to their CD3–TCR complex to mediate lysis of Fc receptor (FcR)-bearing or covalently bound antigen-negative tumor targets in a major histocompatibility complex (MHC)-unrestricted manner (9–12). In the present study, we investigated the capacity of mAbs against a variety of CD antigens to induce such redirected lysis by the CTLs and observed that only those mAbs directed against CD44 or gp90^{MEL-14} molecules expressed on the activated CTLs could evoke the lytic potential. These data suggest that lymphocyte homing receptors may also play a major role in the activation of the cytolytic properties of CTLs.

MATERIALS AND METHODS

Mice. Adult female C57BL/6 (H-2^b) and DBA/2 (H-2^d) mice used in this study were purchased from the National Institutes of Health, Bethesda, MD.

mAbs. The mAbs used were in culture supernatants and were from the following hybridomas: 9F3 (rat IgG; kindly provided by F. J. Dumont, Merck, Sharp & Dohme Research Laboratories, Rahway, NJ), directed against CD44 (Ly-24, Pgp-1) (13, 14); MEL-14 (rat IgG), directed against lymphocyte homing receptor for endothelium (gp90^{MEL-14}) (8); KM703 (rat IgG), against mouse CD44 (Pgp-1) (7); 2.4G2 (rat IgG), against FcR; H57-597 (hamster IgG; gift from R. T. Kubo, National Jewish Center, Denver, CO), against pan anti-$\alpha\beta$TCR (15); 145.2C11 (hamster IgG; kindly provided by J. A. Bluestone, University of Chicago), against CD3 (12); 6B2 (rat IgG), against CD45R; M17/4 (rat IgG), against CD11a [lymphocyte function-associated antigen 1 (LFA-1)]; and 53-6.72 (rat IgG), against CD8. All hybridomas unless otherwise mentioned were obtained from the American Type Culture Collection. mAbs were concentrated by ultrafiltration (Amicon) and used as described in detail (13). F(ab')$_2$ fragments of anti-CD44 and anti-$\alpha\beta$TCR mAbs were prepared from the concentrated mAb preparation by treatment with pepsin (16). Briefly, the mAbs were incubated with pepsin at a pepsin/mAb ratio of 1:100 (vol/vol) in sodium

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Abbreviations: CTL, cytotoxic T lymphocyte; FcR, Fc receptor; mAb, monoclonal antibody; TCR, T-cell receptor; MHC, major histocompatibility complex; LFA-1, lymphocyte function-associated antigen 1; FITC, fluorescein isothiocyanate; MLR, mixed lymphocyte reaction; Pgp-1, phagocytic glycoprotein 1.

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citrate (pH 3.9) for 4 hr at 37°C. The solution was normalized to pH 7 with 3 M Tris·HCl (pH 8.6) and was dialyzed into borate-buffered saline (pH 8.5). The $F(ab')_2$ fragments were passed over a protein A column. The purity of $F(ab')_2$ fragments was indicated by their capacity to bind to CTLs as analyzed by flow cytometry and by their inability to induce redirected lysis.

Flow Cytometry. Cells were analyzed phenotypically using fluorescein isothiocyanate (FITC)-labeled mAbs as described (13, 17). Briefly, cells were first incubated on ice for 30 min with antibodies directed against CD3, $\alpha\beta$TCR, CD8, CD44, CD45R, LFA-1, or FcR. After washing, FITC-conjugated secondary antibody was added, which consisted of anti-rat IgG $F(ab')_2$ or FITC-conjugated anti-hamster IgG $F(ab')_2$ (Cappel Laboratories), depending on the type of primary antibody used. Negative controls included cells incubated with normal rat IgG (for CD8, CD44, CD45R, or LFA-1) or normal hamster IgG (for CD3 and $\alpha\beta$TCR) or medium (for FcR), followed by the corresponding FITC-conjugated secondary antibody. The analysis was performed using a flow cytometer, the Epics V model 752 (Coulter) (13, 17). The total number of cells counted for each sample was 10,000.

Effector Cells. CTL clone PE-9 was used as the effector. This clone was isolated from a C57BL/6 mouse that after treatment with a nitrosourea (18–20) was rejecting LSA, a syngeneic T-cell lymphoma. The clone was obtained from T cells purified from the peritoneal cavity and cultured at 3×10^6 cells per well in the presence of 1×10^5 irradiated LSA tumor cells and recombinant interleukin 2 (50 units/ml; kindly provided by C. Reynolds, National Cancer Institute) in 24-well tissue culture plates in RPMI 1640 medium (GIBCO) supplemented with 2 mM L-glutamine, 50 μM 2-mercaptoethanol, gentamicin (50 μg/ml), and 10% (vol/vol) fetal calf serum (Biocell Laboratories). After long-term culture, the T cells were cloned by limiting dilution (21) and maintained by repeated subculture twice a week. In some assays, CTLs were generated in primary allogeneic mixed lymphocyte reaction (MLR) cultures by incubating purified T cells from the lymph nodes of C57BL/6 mice (3×10^6 cells per well) with irradiated (2000 rads; 1 rad = 0.01 Gy) allogeneic DBA/2 spleen cells (3×10^6 cells per well) in 2 ml of medium in 24-well tissue culture plates. After a 5-day incubation at 37°C, the cells were harvested, and live cells were isolated by centrifugation on Ficoll/Hypaque (Sigma) and tested for CTL activity against P815 ($H\text{-}2^d$) or EL-4 ($H\text{-}2^b$) target cells in the presence or absence of anti-CD44 mAb.

Cytotoxicity Assay. Cytotoxicity was studied by using a ^{51}Cr-release assay (18). Tumor cells EL-4, LSA, P815, and YAC-1, used as target cells, were grown *in vitro* as described (18). Various numbers of effector cells in 0.05 ml were seeded in triplicate into wells of a 96-well round-bottom microtiter plate (Flow Laboratories). To this, 0.05 ml of the appropriate dilution of the mAb or culture medium was added. Tumor targets were labeled with ^{51}Cr by incubating tumor cells in 0.5 ml of medium with 200 μCi of ^{51}Cr as sodium chromate (specific activity, 200–500 Ci/g; 1 Ci = 37 GBq; New England Nuclear) at 37°C for 1 hr. The tumor cells were washed thrice and adjusted to 1×10^5 cells per ml, and 0.1 ml of the labeled targets was added to each well. The plates were incubated for 4 hr at 37°C. The supernatants were then collected using a Skatron harvesting system (Skatron, Sterling, VA), and radioactivity was measured with a γ counter (TMAnalytic, Elk Grove Village, IL). Percent cytotoxicity was calculated from the ^{51}Cr released as follows: [(experimental release − control release)/(total release − control release)] × 100. Control release was measured by incubating ^{51}Cr-labeled targets alone or in the presence of the specific mAb, which was usually >15% of the total ^{51}Cr released. Total release was determined by incubating labeled tumor cells with SDS.

RESULTS AND DISCUSSION

CTL clone PE-9, used in this study, was isolated from nitrosourea-treated C57BL/6 mice (18–20) that were rejecting the syngeneic lymphoma LSA. This T-cell clone specifically mediated lysis of LSA tumor targets but not the heterologous syngeneic tumor target EL-4 or the allogeneic natural killer-sensitive target YAC-1 (Fig. 1*A*). When phe-

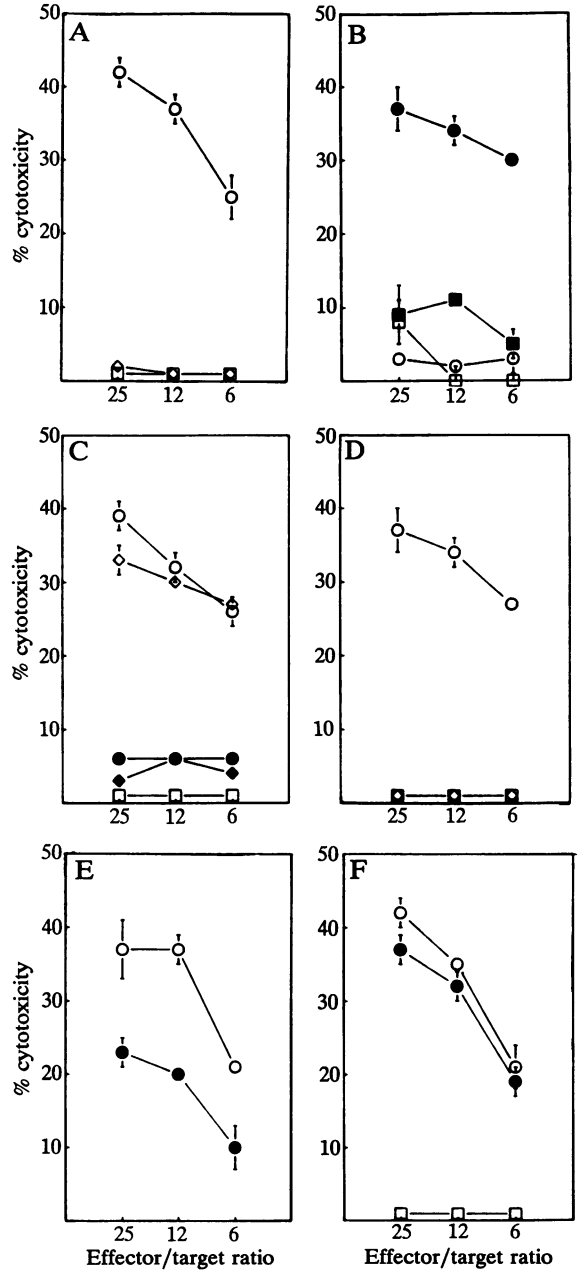

FIG. 1. Cytotoxicity caused by CTL clone PE-9 against various tumor targets in the presence or absence of mAbs against CD44 molecules. PE-9 CTLs were mixed with a variety of ^{51}Cr-labeled tumor targets in the presence or absence of mAbs, and the percent cytotoxicity was measured by a ^{51}Cr-release assay. The anti-CD44 mAb used was obtained from the 9F3 hybridoma. All mAbs were concentrated by ultrafiltration (Amicon) and used at a final dilution of 1:4. (*A*) □, EL-4; ○, LSA; ◇, YAC-1. (*B*) ○, EL-4; ●, EL-4 plus anti-CD44; □, YAC-1; ■, YAC-1 plus anti-CD44. (*C*) □, EL-4; ○, EL-4 plus anti-CD44; ●, EL-4 plus anti-CD44 $F(ab')_2$; ◇, EL-4 plus anti-$\alpha\beta$TCR; ◆, EL-4 plus anti-$\alpha\beta$TCR $F(ab')_2$. (*D*) □, EL-4; ○, EL-4 plus anti-CD44; ◇, EL-4 plus anti-CD44 plus anti-FcR. (*E*) ○, LSA; ●, LSA plus anti-CD44 $F(ab')_2$. (*F*) □, EL-4; ○, EL-4 plus anti-CD44; ●, EL-4 plus anti-CD44 plus anti-$\alpha\beta$TCR $F(ab')_2$.

notypically characterized, this clone was found to be $\alpha\beta$TCR$^+$, CD8$^+$, CD3$^+$, CD44$^+$, MEL-14$^+$, CD45R$^+$, CD11a (LFA-1$^+$), and FcR$^-$ (Figs. 2 and 3).

Recent studies have shown that anti-TCR antibodies can trigger the lytic activity of CTLs and that this activity can be redirected to an antigen-negative nonspecific target cell by inducing conjugate formation between the effector and target cells (9–12). Thus, mAbs against the CTL TCR can induce lysis of any nonspecific target cell bearing the FcR. As EL-4 cells were strongly FcR$^+$ (Fig. 3) but lacked the determinant recognized by the TCR of PE-9 CTLs (Fig. 1*A*), we considered EL-4 cells to be ideal target cells to study the redirected lysis mediated by PE-9 CTLs and induced by mAbs against a variety of CD antigens. In addition, we also included YAC-1 tumor targets because these cells lacked the FcR (Fig. 3) and were resistant to PE-9-mediated lysis (Fig. 1*A*). To address whether mAbs against the CD molecules expressed by CTL clone PE-9 could trigger the CTL lytic activity, PE-9 CTLs were mixed with ^{51}Cr-labeled EL-4 targets at various effector/target ratios and with various mAbs. A representative experiment, depicted in Fig. 4, demonstrated that mAbs against $\alpha\beta$TCR or CD3 could activate CTLs to mediate lysis of EL-4 target cells. Interestingly, mAb 9F3, against CD44, could also induce efficient lysis of EL-4 cells. In contrast, mAbs against CD45R, CD8, CD11a (LFA-1), or normal rat IgG (control) failed to activate the lytic functions of the CTLs even at saturating concentrations (Fig. 4).

Several experiments were carried out to substantiate that the CTL-mediated lysis induced by anti-CD44 mAbs resulted from conjugate formation between the effector and target cells in which anti-CD44 mAbs bound to the EL-4 targets by the Fc region and bound to the effector CTLs by the Fab region. For example, in the presence of anti-CD44 mAbs, PE-9 CTLs lysed the FcR$^+$ EL-4 targets but not FcR$^-$ YAC

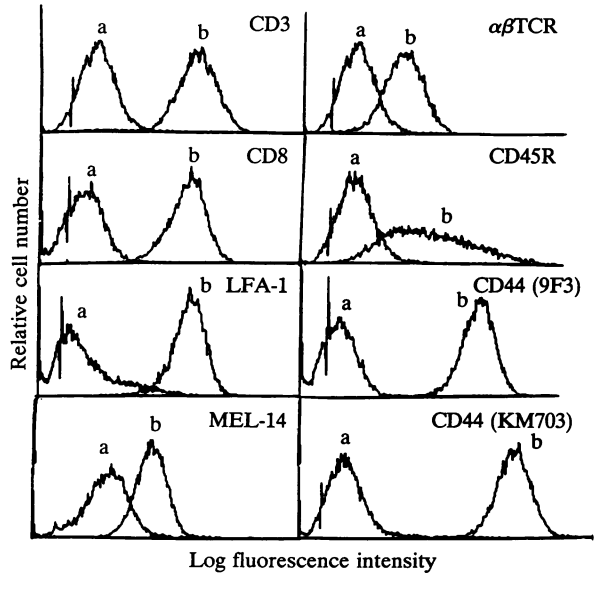

FIG. 2. Analysis of the surface phenotype of CTL clone PE-9 by flow cytometry. Fluorescence profiles depict negative controls (peaks a) and cells stained with various antibodies (peaks b). Hybridomas used include 145.2C11 (anti-CD3, hamster IgG), H57-597 (anti-$\alpha\beta$TCR, hamster IgG), 53-6.72 (anti-CD8, rat IgG), 6B2 (anti-CD45R, rat IgG), M17/4 (anti-LFA-1, rat IgG), 9F3 (anti-CD44, rat IgG), MEL-14 (gp90^{MEL-14}, rat IgG), and KM703 (anti-CD44, rat IgG). The secondary antibody used consisted of FITC-conjugated anti-rat IgG F(ab')$_2$ or FITC-conjugated anti-hamster IgG F(ab')$_2$, depending on the type of primary antibody. Negative controls included cells incubated with normal rat IgG (for CD8, CD44, CD45R, MEL-14, and LFA-1) or normal hamster IgG (for CD3 and $\alpha\beta$TCR), followed by the corresponding FITC-conjugated secondary antibody. Analysis was performed using a flow cytometer.

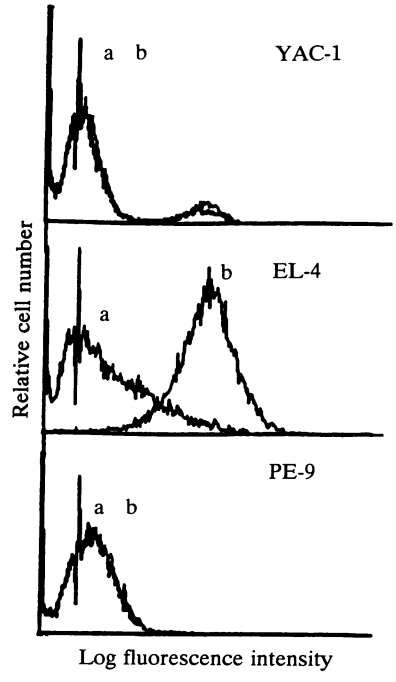

FIG. 3. FcR expression by CTL clone PE-9 and by tumor cells YAC-1 and EL-4. Fluorescence profiles depict negative controls (peaks a) and cells stained with anti-FcR mAb (peaks b). Cells were stained with anti-FcR mAb (2.4G2, rat IgG) followed by FITC-conjugated anti-rat IgG F(ab')$_2$. The cells were analyzed as described in Fig. 2.

targets (Fig. 1*B*). Furthermore, F(ab')$_2$ fragments of anti-CD44 or anti-$\alpha\beta$TCR mAbs failed to induce significant lysis of EL-4 cells (Fig. 1*C*), although they could efficiently bind to PE-9 CTLs, as determined by flow cytometry (data not shown). Also, addition of mAbs (2.4G2) against the murine FcR could inhibit the CD44-mediated lysis of EL-4 cells (Fig. 1*D*). Lastly, F(ab')$_2$ fragments of anti-CD44 mAbs blocked the FcR-dependent lysis induced by the intact anti-CD44 mAbs (data not shown).

To study the role played by CD44 in the antigen-specific PE-9-mediated lysis, F(ab')$_2$ fragments of anti-CD44 mAbs were added to the cytotoxicity assay. The data in Fig. 1*E* show that F(ab')$_2$ fragments of anti-CD44 mAbs significantly inhibited PE-9-mediated lysis of antigen-specific LSA targets. These data suggest that CD44 may play a role in TCR-mediated CTL activation. Because EL-4 cells lack the

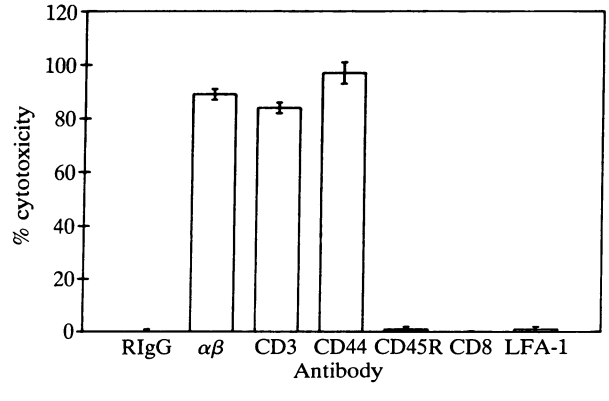

FIG. 4. Cytotoxicity caused by CTL clone PE-9 against EL-4 target cells in the presence of normal rat IgG (RIgG, control) or mAb against a variety of surface markers expressed by the CTL clone. The anti-CD44 mAb used was obtained from the 9F3 hybridoma. The cytotoxicity depicted was obtained at an effector/target ratio of 50:1 by using the ^{51}Cr-release assay. $\alpha\beta$, $\alpha\beta$TCR.

antigenic determinant recognized by the TCR of PE-9 CTLs, CD44-mediated lysis was probably independent of TCR-mediated CTL activation. To further corroborate this finding, $F(ab')_2$ fragments of anti-$\alpha\beta$TCR mAbs were added to the cytotoxicity assay, in which anti-CD44 mAbs redirected the lysis of EL-4 target cells. As seen in Fig. 1*F*, anti-$\alpha\beta$TCR $F(ab')_2$ fragments failed to significantly inhibit CD44-mediated lysis, thereby suggesting that the CD44-mediated lysis was independent of the TCR.

Recently, several mAbs directed against various CD44 epitopes have been found to differ with respect to their functional efficiency (7). In the present study, we therefore compared three mAbs, 9F3, MEL-14, and KM703, for their capacity to activate lytic functions of the CTLs. mAb 9F3 is directed against the mouse Pgp-1 (CD44). mAb MEL-14, directed against the murine lymphocyte homing receptor for high endothelial venules, precipitates a protein of ≈90 kDa that has been designated $gp90^{MEL-14}$ (22). The human Hermes molecule involved in the lymphocyte homing to high endothelial venules has been found to be indistinguishable from CD44 (Pgp-1) (23). Although human Hermes molecule and mouse $gp90^{MEL-14}$ are structurally distinct (24, 25), mAb MEL-14 recognizes human Hermes/CD44. Furthermore, the surface expression of $gp90^{MEL-14}$ and human CD44 (Pgp-1) on lymphocytes appears to relate to the migratory capability and the activation state of the cells (26). For these reasons, we included mAb MEL-14 in the current study. Lastly, mAb KM-703 was shown to react with mouse CD44 (Pgp-1) and to block lympho-hemopoiesis in long-term bone marrow cultures (7).

When cultured PE-9 CTLs were stained with various mAbs, a high density of CD44 antigen expression on the CTLs was observed with mAbs KM-703 and 9F3, whereas a lower but significant density was observed with mAb MEL-14 (Fig. 2). Interestingly, however, when all three mAbs were compared for their capacity to induce redirected lysis by PE-9 CTLs, mAbs 9F3 and MEL-14 induced strong and comparable lysis, whereas mAb KM-703 even at saturating concentrations evoked only marginal lytic activity (Fig. 5*A*). Miyake *et al.* (7) compared mAbs MEL-14 and KM-703 for their capacity to block lympho-hemopoiesis in long-term bone marrow cultures and found that KM-703 was suppressive, whereas MEL-14 was not. Thus, it is likely that these mAbs recognize distinct epitopes. It should also be noted that T cells from resting lymph nodes expressed a lower density of CD44 antigen than T cells activated with concanavalin A or through the TCR (data not shown).

Further studies were carried out to investigate whether the CD44-mediated lysis was restricted to the CTL clone PE-9 or whether this property could be generalized to all CTLs. Thus we generated alloreactive CTLs in primary MLR cultures by growing purified lymph node T cells from C57BL/6 ($H-2^b$) mice with irradiated spleen cells from DBA/2 ($H-2^d$) mice. After a 5-day incubation, the cultures were harvested and the CTL activity generated was tested against ^{51}Cr-labeled P815 ($H-2^d$) targets or syngeneic EL-4 ($H-2^b$) targets. The EL-4 targets were incubated in the presence or absence of anti-CD44 mAb. The data in Fig. 5*B* demonstrated that the CTLs generated in primary MLR cultures could lyse only the specific target P815, not the syngeneic target EL-4. However, in the presence of anti-CD44 mAb, the primary CTL cultures could mediate strong lysis of EL-4 targets. We have observed that resting T cells express CD44. We therefore investigated whether anti-CD44 mAb could trigger the lytic activity in resting CTLs. Thus purified T cells from C57BL/6 lymph nodes were tested for lytic activity against P815 or EL-4 targets as described above. The data demonstrated that unactivated T cells lysed neither P815 targets nor EL-4 targets, even in the presence of anti-CD44 mAbs (Fig. 5*B*).

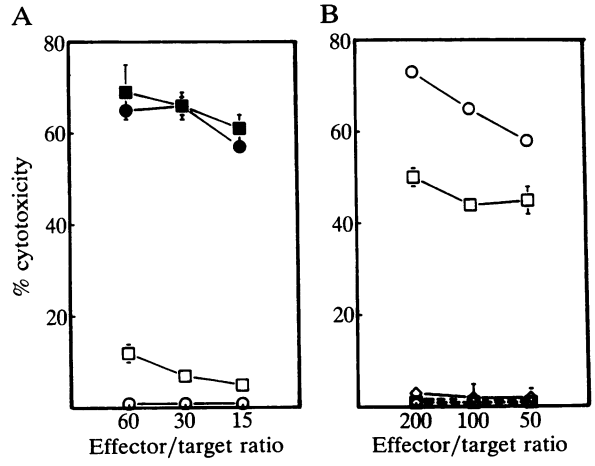

FIG. 5. Analysis of CTL activity mediated by CD44 using CTL clone PE-9 and CTLs generated in primary allogeneic MLR culture. (*A*) PE-9 CTLs were tested for lytic activity against EL-4 targets in the absence or presence of mAbs 9F3, KM703, and MEL-14, similar to the analysis described in Fig. 1. ○, EL-4; ●, EL-4 plus 9F3; □, EL-4 plus KM703; ■, EL-4 plus MEL-14. (*B*) Solid lines represent CTLs generated in primary allogeneic MLR culture by culturing T cells from lymph nodes of C57BL/6 mice ($H-2^b$) with irradiated DBA/2 ($H-2^d$) spleen cells for 5 days, and the CTL activity was tested against P815 ($H-2^d$) or EL-4 ($H-2^b$) targets. Broken lines represent freshly isolated T cells from the lymph nodes of C57BL/6 mice directly tested for lysis against P815 or EL-4 targets. The EL-4 targets were tested in the presence or absence of anti-CD44 mAb (9F3). ○, P815; ◇, EL-4; □, EL-4 plus anti-CD44.

Often, activation of $CD3^+$ T cells leads to MHC-unrestricted lysis of target cells (for review, see ref. 27). Because addition of mAbs against a variety of cell surface proteins, such as CD11a/CD18 (LFA-1), CD2 (LFA-2), and CD45, inhibits the MHC-unrestricted cytotoxicity, it was hypothesized that lysis of nonspecific targets by activated CTLs can be triggered by signals delivered through any of the several CD antigens after their interaction with the ligands expressed on target cells (27). However, by using redirected lysis, the current study and earlier studies (12) demonstrated that CD8, Thy-1, Ly-6.2c, CD11a/CD18 (LFA-1), CD45, and class I MHC antigens expressed by the CTLs fail to deliver the transmembrane signals necessary to release the lytic granules and mediate target-cell lysis. It should be noted that pairs of mAbs directed against two distinct epitopes on the CD2 antigen, but not anti-CD2 mAbs directed against a single epitope, have been shown to trigger cytolytic activity of human CTLs (28). Thus, of the cell surface determinants so far studied, CD44 and $gp90^{MEL-14}$ may play a major role in MHC-unrestricted cytotoxicity.

Recent studies have suggested that there may be multiple ligands for CD44 (1). The CD44 molecule has been shown to bind to specific ligands on endothelial cells, termed vascular addressin (29, 30), and this interaction may provide a basis for the lymphocyte homing to lymphoid tissues (1). Furthermore, anti-CD44 mAbs have been shown to promote cell adhesion and to augment the proliferation of human peripheral blood T cells induced by CD2 or CD3 antibodies (1, 4–6, 31). In this context, our studies raise the question of whether the interaction between CD44 or $gp90^{MEL-14}$ molecules, expressed on activated CTLs, and their respective ligands, expressed on antigen-negative target cells (such as endothelial cells), *in vivo*, is sufficient to activate the nonspecific lysis of the target cells. The extent of such TCR-independent lysis may depend on the density of CD44 antigen expression and may be regulated by other interacting adhesion molecules such as CD2 and CD11a/CD18 (LFA-1). Furthermore, because the CD44 molecule has been reported to be present in

serum (32), it is possible that this may downregulate T-cell activity. If CD44-mediated TCR-independent CTL-mediated lysis does occur *in vivo*, it can contribute to the nonspecific tissue damage associated with CTL-mediated inflammation. For example, $CD8^+$ CTLs have been implicated in the massive inflammation in the central nervous system of mice carrying lymphocytic choriomeningitis virus (for review, see ref. 33). The pathogenesis seems to result from the destruction of endothelial cells located at the blood–cerebrospinal fluid barrier by the $CD8^+$ CTLs. Because such CTLs are strongly $CD44^+$ and endothelial cells bear the CD44 ligands, it is tempting to speculate that the destruction of endothelial cells may also result from direct T-cell lytic activity mediated by CD44. Also, it is likely that the interaction between interleukin 2-activated lymphoid cells and endothelial cells may be responsible for vascular leakage syndrome and systemic toxicity associated with administration of high doses of interleukin 2 used in the immunotherapy of cancer (34). The current study demonstrates that CD44 and $gp90^{MEL-14}$ molecules, in addition to participating in T-cell homing, activation, and adhesion, may play a major role in directly delivering the transmembrane signal to the CTLs that induces lytic activity.

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