Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6949–6953, September 1990
Immunology

T-cell receptor-negative natural killer cells display antigen-specific cytotoxicity for microvascular endothelial cells

(allospecificity/antigen receptor/transplantation)

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Communicated by George E. Palade, May 9, 1990

ABSTRACT **Based upon prior demonstrations that human microvascular endothelial cells (ECs) could serve as natural killer (NK) cell targets, we established NK cell lines and clones by repeated stimulation of highly purified CD16-positive, CD3/T-cell receptor (Ti)-negative cells with allogeneic ECs. After 3 weeks in culture these lymphoid cells, which neither expressed surface CD3/Ti molecules nor rearranged Ti β- or γ-chain genes and which lysed K562 human erythroleukemia cells, displayed specific cytotoxicity for the stimulating ECs. Furthermore, freshly isolated NK cells bound and then removed from each of several allogeneic EC lines displayed selective cytotoxicity for the adsorbing EC line. These results provide evidence for alloantigen-specific recognition of microvascular ECs by NK cells that appears to be determined, at least in part, at the level of adherence. We discuss the implications of these findings with respect to the rejection of vascularized organ allografts.**

Natural killer (NK) cells are a subpopulation of lymphocytes that lyse susceptible targets, such as transformed or virally infected cells, without apparent prior sensitization and with no known specificity for antigen or restriction for gene products of the major histocompatibility complex (MHC) (1–3). The ability of NK cells to lyse a wide range of cell types, combined with the fact that NK cells do not utilize the CD3/T-cell receptor (Ti) complex to recognize their targets, has led to the speculation that such effector cells recognize a widely distributed antigen or, alternatively, lack any antigen specificity whatsoever.

We previously explored the interaction between NK cells and allogeneic microvascular endothelial cells (ECs) in an effort to determine whether NK–EC interactions play a role in organ allograft rejection. $CD16^+$ (NK) cells preferentially bound and induced the expression of MHC class II antigens on such ECs (4, 5) and promoted striking morphological alterations in EC monolayers (6). In the current study we sought to determine whether the interaction between NK cells and ECs is antigen-specific. CD3/Ti-negative lymphocytes that had phenotypic and functional properties of NK cells, propagated in the presence of microvascular EC and recombinant interleukin 2 (rIL-2), were capable of lysing their EC stimulators in an antigen-specific manner. This suggests the presence of a novel "antigen receptor" that, based upon these studies, may recognize a set of polymorphic EC antigens, distinct from human leukocyte antigens (HLA).

MATERIALS AND METHODS

Microvascular EC Culture. Stable cultures of human microvascular ECs were established by trypsinization of preputial skin from anonymous newborns as described (4). Stable cultures of autologous fibroblasts were established by plating a small aliquot of cells obtained from the EC isolation procedure into a 35-mm dish in the presence of 10% human peripartum serum but without EC selection components (4). Also, B-lymphoblastoid cell lines (LCLs) autologous to a given EC line were established by Epstein–Barr virus transformation of the relevant cord blood lymphocytes (7). The resulting stable lines were $CD19^+$ and used as LCL targets syngeneic to the isolated EC line.

Monoclonal Antibodies (mAbs). Anti-intercellular adhesion molecule 1 (anti-ICAM-1, clone RR 1.1, IgG1) was a gift from R. Rothlein (Boehringer Ingelheim, Ridgefield, CT). Anti-CD44 (clone 515, IgG2a) reacts with a lymphocyte homing receptor molecule expressed on all leukocytes (8) and was kindly provided by G. Kansas (University of Iowa). WT31 (IgG1) recognizes a monomorphic determinant on the αβ Ti complex and was purchased from Becton Dickinson. All the other mAbs have been described in detail (5).

Lymphocyte Lines. Peripheral blood lymphocytes (PBLs) were isolated by Ficoll/Hypaque gradient centrifugation and depleted of monocytes by plastic adherence and subsequent passage over nylon wool columns. $CD16^+$ cells were positively selected by a panning technique (9) with anti-Leu-11c. $CD16^+$ cells were further depleted of T cells by sorting the negative population after treatment with a mixture of anti-Leu-4 and WT31 antibodies, plus fluorescein-conjugated goat anti-mouse IgG as a second step. The resultant $CD16^+$ population was <0.1% contaminated by $CD3/Ti^+$ cells. Control T-cell populations were obtained by panning $CD16^-$ cells with anti-Leu-3a antibody and recovery of the negatively selected cells, which were <1% $CD16^+$ and 85–95% $CD8^+$. The $CD16^+$ or control T-cell populations were cocultured with confluent EC monolayers in 75-cm^2 flasks in the presence of culture medium containing 10% pooled human serum, 150 units of rIL-2 per ml, and 20% LCL-conditioned medium generated as described (10). In some experiments, the purified lymphocytes were divided into two fractions and stimulated by two different EC lines. Cultures were refed every 4–6 days with the same culture medium. After 3 weeks, the lymphocytes, which in the case of the $CD16^+$ populations were mostly EC-adherent, were recovered with 1% EDTA, with termination of the recovery before the ECs detached.

After confirmation of their antigen specificity, some lines were replated at 0.7 cell per well into 96 microwells containing confluent monolayers of the stimulating EC line and were refed every 4 days with the rIL-2-containing medium outlined

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Abbreviations: EC, endothelial cell; Ti, T-cell receptor; NK, natural killer; MHC, major histocompatibility complex; LFA-1, lymphocyte function-associated antigen 1; PBL, peripheral blood lymphocyte; rIL-2, recombinant interleukin 2; mAb, monoclonal antibody; ICAM, intercellular adhesion molecule; LCL, lymphoblastoid cell line; E/T, effector/target.

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above and weekly with 10^4 irradiated [3000 rads (30 Gy)] ECs. After 4 weeks, sufficient numbers of cloned cells were present for functional testing.

Lymphocyte EC Cytotoxicity and Binding Assays. Monocyte-depleted PBLs, recovered EC-adherent lymphocytes, propagated lymphocyte lines, or clones were used as effectors in 4-hr ^{51}Cr-release assays in microtiter wells at the indicated effector/target (E/T) ratios. ECs and fibroblasts were labeled with 2 μCi (74 kBq) of ^{51}Cr per well and used as adherent targets in confluent monolayers (4). The erythroleukemia line K562 and Epstein–Barr virus-transformed B lymphoblasts were labeled with 50 μCi per 10^6 cells and used as targets in suspension.

Because of the well-described enhancement of NK activity by IL-2 (11), monocyte-depleted PBLs were incubated for 18 hr at 37°C with rIL-2 (Cetus; 20 units/ml) before their use as effectors in some experiments. These IL-2-treated cells were added to confluent EC monolayers in 75-cm^2 Falcon flasks and allowed to bind for 90 min at 37°C, after which nonadherent lymphocytes were removed by repeated monolayer washing and adherent lymphocytes were removed by 1% EDTA treatment (4). The recovered EC-adherent lymphocytes were then used as effectors, with labeled EC targets from the same EC line used for the initial selection (binding) or from two irrelevant EC lines.

The propagated lymphocyte lines or clones were recovered as described above and used as effectors in 4-hr ^{51}Cr-release assays with the relevant, stimulating EC line, irrelevant EC lines, and a variety of control lines as targets. In some experiments, mAbs were added at 10–25 μg/ml directly to the cytotoxicity assay mixtures to address the ability of the antibodies to block the observed killing. Percent ^{51}Cr release (percent specific cytotoxicity) was determined as described (4).

Lymphocyte–EC binding was evaluated in a 90-min assay at 4:1 lymphocyte/EC ratios (4).

Southern Blot Analysis. DNA was extracted as described (12). In brief, 5–10 × 10^6 cells were incubated overnight at 37°C in 0.5% SDS with proteinase K at 0.2 mg/ml, followed by phenol/chloroform extraction and ethanol precipitation. DNA was digested with the indicated restriction enzymes, size-fractionated by agarose gel electrophoresis, and transferred onto nitrocellulose by the method of Southern (13). Filters were hybridized to nick-translated, ^{32}P-labeled probes and washed at 55°C in 0.1% SDS/0.15 M NaCl/0.0015 M sodium citrate, pH 7, prior to autoradiography. Probes used were (*i*) a 1.0-kilobase (kb) *Bgl* II–*Bgl* II fragment from $C_{\beta 2}$ (which hybridizes equally to the Ti β-chain constant-region gene segments $C_{\beta 1}$ and $C_{\beta 2}$) and (*ii*) a 310-base-pair cDNA fragment specific for $C_{\gamma 1}$ and $C_{\gamma 2}$, as previously described (14). Both probes were generously provided by N. Berliner (Yale University).

RESULTS AND DISCUSSION

In initial studies, monocyte-depleted PBLs from healthy donors were cultured overnight in IL-2-containing medium and then added to three different allogeneic EC lines. After a 90-min coincubation, the EC-adherent lymphocytes were recovered and further analyzed. As previously described (4), these cells were highly enriched for cells of NK phenotype (CD3$^-$ CD16$^+$) (data not shown). When used as effectors in 4-hr cytotoxicity assays, they were cytotoxic for ECs (Fig. 1). Furthermore, lymphocytes adherent to each of three allogeneic EC lines demonstrated preferential cytotoxicity against the line to which they bound (Fig. 1). Although this result was highly reproducible, it was nonetheless surprising since, except for the 90-min coincubation with ECs, the effector cells had not been previously exposed to their targets.

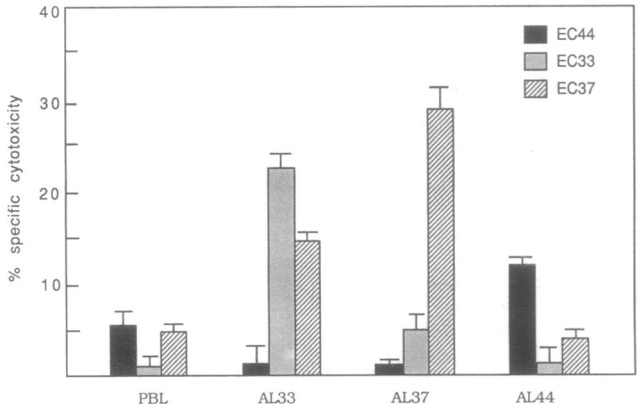

FIG. 1. Cytotoxic activity against three different EC lines (EC44, EC33, and EC37) by monocyte-depleted PBLs and EC-adherent lymphocytes (AL) recovered after a 90-min binding of PBLs to EC33 (AL33), EC37 (AL37), and EC44 (AL44).

To explore the possibility that these effectors might be expandable *in vitro* as cell lines, CD16$^+$ CD3$^-$ lymphocytes were highly purified by a combination of panning and sorting and were cocultured for 3 weeks with one of three allogeneic microvascular EC lines (EC45, EC158, or EC178) in the presence of IL-2-containing conditioned medium. These lymphocyte populations underwent up to 5-fold expansions during the 3-week culture period and not only adhered rapidly when initially added to the EC monolayers but continued to grow in an EC-adherent fashion. The expansion of these lymphoid cells was dependent on the continued presence of EC monolayers, as the same cells cultured in parallel in identical medium but without ECs grew minimally. Surface immunofluorescent staining of the propagated lines demonstrated that they were WT31$^-$ and CD3$^-$, 90–96% CD2$^+$, 48–56% CD8^{dull+}, and 70–90% CD16$^+$ (data not shown). T cells obtained from the donors of the CD16$^+$ cytolytic lines also proliferated in the presence of allogeneic EC monolayers and conditioned medium. However, these cells did not grow as EC-adherent lines and were >99% CD3$^+$ WT31$^+$ and 90% CD8$^+$ (data not shown).

To rule out the possibility that Ti gene products were responsible for the alloantigen-specific cytotoxicity displayed by our CD3$^-$ NK cell lines, genomic DNA and RNA analyses were performed on representative effector cell lines and clones. Fig. 2*A* shows Southern blot analyses of *Eco*RI and *Hin*dIII DNA digests derived from clone AL181d and controls, with the C_β cDNA probe. These restriction enzyme digests were chosen for their ability to detect rearrangements involving $C_{\beta 1}$ and $C_{\beta 2}$ genes, respectively (15). Similarly,

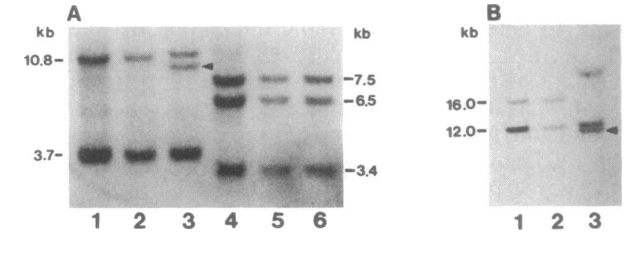

FIG. 2. Southern blot analysis of the antigen-specific NK clone 181d (CD3$^-$ CD16$^+$) (*A* lanes 1 and 4; *B* lane 1), the B-LCL Arent (negative control) (*A* lanes 2 and 5; *B* lane 2) and the T-cell line HPB-ALL (positive control) (*A* lanes 3 and 6; *B* lane 3). ^{32}P-labeled probes used were specific for the Ti β-chain (*A*) or γ-chain (*B*) gene. DNA was digested with *Eco*RI (*A* lanes 1–3), *Hin*dIII (*A* lanes 4–6), or *Bam*HI (*B*). The sizes of the expected germ-line C_β and C_γ fragments are indicated. Arrows denote the presence of rearranged bands. The high molecular weight fragment in *B* lane 3 represents partial digestion.

Fig. 2*B* shows *Bam*HI digests derived from the same clone and hybridized with the C_γ cDNA probe. Rearrangements of Ti β and γ genes were detected in genomic DNA digests from the T-cell leukemia line HPB-ALL (Fig. 2, lanes 3), as previously described (16). However, these Ti loci were in germ-line configuration in the NK clone analyzed (Fig. 2*A*, lanes 1 and 4, and Fig. 2*B*, lane 1), identical to the control B-LCL (Fig. 2*A*, lanes 2 and 5, and Fig. 2*B*, lane 2). *Hin*dIII digests also failed to show Ti γ-gene rearrangement (data not shown). These results make it unlikely that Ti $\alpha\beta$ or $\gamma\delta$ heterodimers could be functionally expressed on these cells.

Furthermore, no Ti β or γ messages were detected in Northern blot analyses of total cellular RNA from antigen-specific $CD3^-$ $CD16^+$ cell lines (data not shown), a result that is not surprising in view of the lack of gene rearrangement and detectable surface CD3 molecules.

The $CD3/Ti^-$ $CD16^+$ cell lines were evaluated for their ability to lyse the relevant, stimulating EC line or irrelevant lines in 4-hr ^{51}Cr-release assays. AL45 was highly cytotoxic (50% cytotoxicity at a 1:1 E/T ratio) toward EC45 and was unable to kill the two irrelevant lines EC35 and EC129 (Fig. 3*A*). AL158 (Fig. 3*B*) and AL178 (Fig. 3*C*) were derived from the same individual and were stimulated in parallel by EC158 and EC178, respectively. Reciprocal killing experiments were performed to address the possibility that target sensitivity was the major determinant of EC susceptibility to lysis by any of the NK lines. As shown, AL158 and AL178 displayed strongly preferential cytotoxicity toward the relevant stimulating EC line, although AL178 also exhibited detectable lysis of EC158 and EC180 (15.9% and 16.4% cytotoxicity, respectively, at a 1:1 E/T ratio). At E/T ratios higher than 1:1, the extent of nonspecific killing rose, but even at E/T ratios of 25:1, lysis of the stimulating ECs was greater than that of irrelevant ECs (data not shown). These results, consistently obtained in reciprocal killing experiments, make it highly unlikely that quantitative differences in the expression of adhesion structures, and therefore target sensitivity, were responsible for the observed cytotoxic specificity. All three lymphoid lines were highly cytotoxic (>70% cytotoxicity at a 1:1 E/T ratio) to the NK-sensitive K562 erythroleukemia line (data not shown).

To investigate whether this phenomenon was specific to EC targets, fibroblasts were also used as targets in cytotoxicity assays. At the 1:1 E/T ratio, AL45 (Fig. 3*D*), AL158 (Fig. 3*E*), and AL178 (Fig. 3*F*) lysed their stimulating EC lines more efficiently than they did the respective syngeneic fibroblast lines. However, all the lymphocyte lines demonstrated a greater degree of cytotoxicity toward the "relevant" fibroblast line than toward an irrelevant fibroblast line. This raises the possibility that ECs and fibroblasts share a putative allogeneic target molecule(s), which is simply more highly expressed on ECs, or that ECs are more sensitive to lysis.

To assess target sensitivity, freshly isolated peripheral blood mononuclear cells were treated for 5 days with rIL-2 (250 units/ml) and used as effectors in 4-hr ^{51}Cr-release assays. Labeled targets were allogeneic ECs, fibroblasts, and B lymphoblasts. The targets were all derived from the same individual. At a 5:1 E/T ratio, the "lymphokine-activated killer cells" lysed ECs slightly more efficiently than the syngeneic fibroblasts (39% vs. 33%), whereas there was substantially less cytotoxicity against the autologous B-LCL (14%) (data not shown). Thus, IL-2-activated cells can lyse both ECs and fibroblasts efficiently, as shown previously (17). This suggests that the greater degree of killing by the $CD3/Ti^-$ lines against the relevant EC targets, as compared with the syngeneic fibroblast targets, represents a greater or more homogeneous expression of the putative allogeneic target molecule on ECs.

To assess the noted allospecificity at the clonal level, an antigen-specific $CD3^-$ line, AL181, was plated at 0.7 cell per well, expanded, and tested for phenotype and function. The clonally expanded wells contained homogeneous populations that were all $CD3/WT31^-$ by cytofluorography (data not

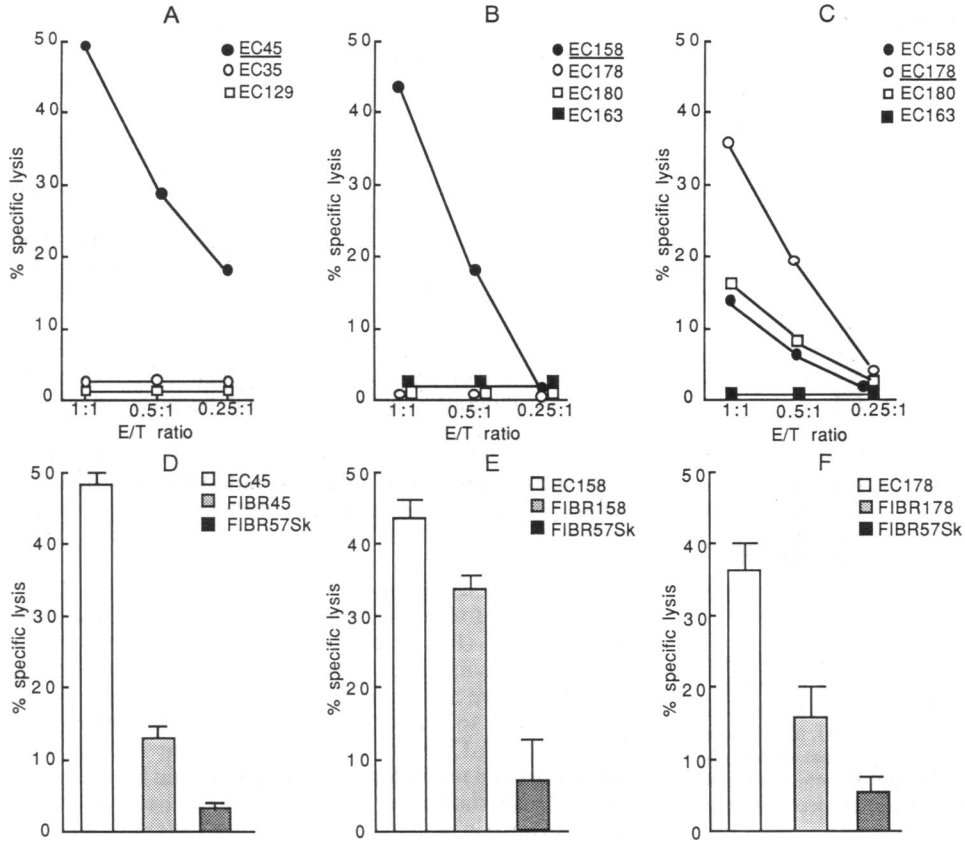

FIG. 3. Cytotoxic activity of NK cell lines AL45 (*A* and *D*), AL158 (*B* and *E*), and AL178 (*C* and F) generated by continuous stimulation with EC lines EC45, EC158, and EC178. AL158 and AL178 were derived from the same individual. (*A–C*) Cytotoxicity against the EC lines used in the sensitization cultures (underlined) and several other EC lines. (*D–F*) Cytotoxicity (E/T ratio 1:1) against skin fibroblasts syngeneic to the stimulator EC lines (FIBR45 in *D*, FIBR158 in *E*, and FIBR178 in *F*) or fibroblasts derived from a genetically unrelated individual (FIBR57Sk). Specific cytotoxicity was evaluated in a 4-hr assay using ^{51}Cr-labeled targets.

shown). Some clones were $CD16^+$ while some were $CD16^-$. Similarly, there were $CD8^+$ and $CD8^-$ clones. Clone 181d, a representative $CD3^-$ $CD16^+$ $CD8^{dull+}$ clone, preferentially lysed the relevant stimulating EC line, with high cytotoxic potential (42% cytotoxicity at a 1:1 E/T ratio; Fig. 4*A*). As with the bulk $CD3^-$ lines, analysis at the clonal level demonstrated preferential cytotoxicity against the relevant fibroblast line, albeit it was less efficiently lysed than its syngeneic EC counterpart (Fig. 4*B*). The B-LCL autologous to the stimulating EC line was not killed by clone 181d. As noted above, B-LCLs are relatively resistant to lysis by IL-2-activated cells. Thus, future definition of the target antigen(s) will be required to assess whether such molecules are not expressed on lymphoid cells.

To examine the role of various cell surface molecules in the lysis of ECs, blocking experiments were performed using a panel of mAbs. For these experiments, mAbs were added at 10–25 μg/ml directly to the cytotoxicity assay wells. As expected, neither anti-Leu-4, which recognizes the CD3 molecule, nor WT31, which recognizes a monomorphic determinant on the $\alpha\beta$ Ti complex, had any effect on these $CD3/Ti^-$ cells (Fig. 5). Similarly, W6/32 antibody failed to inhibit cytotoxicity, suggesting that class I HLA molecules do not serve as the relevant targets on ECs. As the effectors and targets were $CD4^-$ and class II HLA^-, respectively, anti-Leu-3 and CA141 antibodies failed to inhibit lysis, as expected. Despite the fact that a large percentage of cells in the effector population expressed CD8, anti-Leu-2 mAb also did not block killing (Fig. 5). In contrast, anti-lymphocyte function-associated antigen 1 (LFA-1; CD11a is the α subunit) and anti-ICAM-1 consistently inhibited cytotoxicity (>50%), which presumably reflects the role of this adhesion pathway in leukocyte binding to ECs (4, 18, 19). However, it seems unlikely that LFA-1 is responsible for the specificity of cytotoxicity, since in other systems LFA-1-mediated adhesion is not antigen-specific (20). The CD2 molecule has been reported to play an important role in the triggering of early $CD3^-$ stage I and II thymocytes (21), as well as NK clones (22), and on this basis CD2 has been postulated to be the

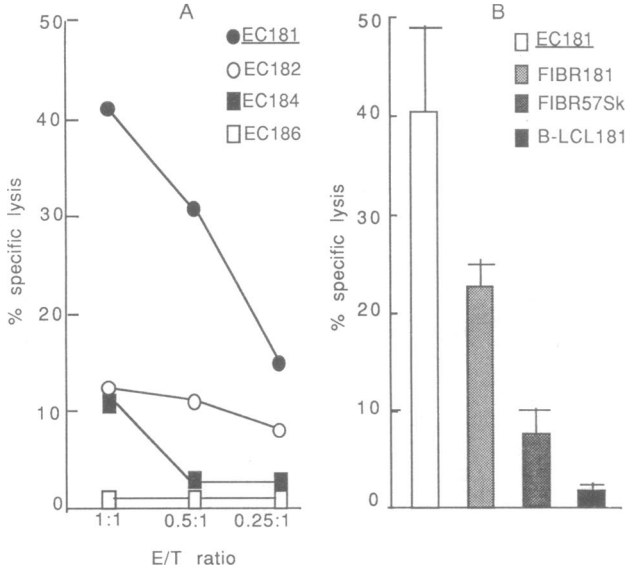

FIG. 4. Cytotoxic activity of NK clone 181d, which is a representative of several clones derived by plating AL181, a $CD3^-$ $CD16^+$ EC181-specific NK line, at 0.7 cell per well. (*A*) Cytotoxicity against the EC line used in the sensitization culture (EC181, ●), and several other EC lines. (*B*) Cytotoxicity (E/T ratio 1:1) against skin fibroblasts and Epstein–Barr virus-transformed lymphoblasts syngeneic to the stimulator EC line (FIBR181 and B-LCL181, respectively) and an irrelevant fibroblast line (FIBR57Sk).

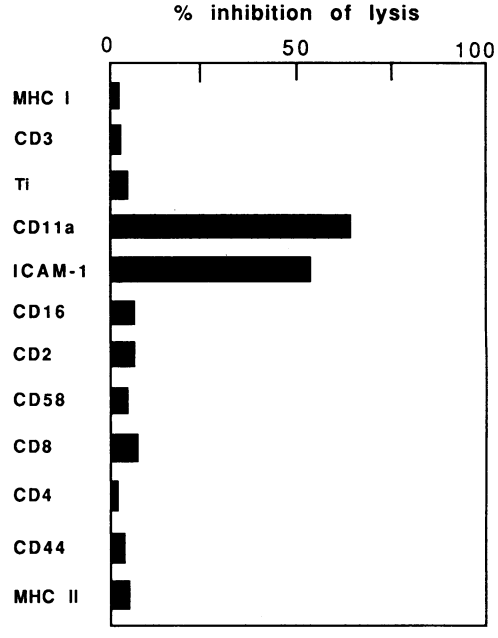

FIG. 5. Inhibition of EC-specific cytotoxic activity of the NK cell line AL158 by a panel of murine mAbs. Specific cytotoxicity at the E/T ratio used (1:1) was 44%. Antibodies to the indicated cell surface antigens were added directly at saturating concentrations (10–25 μg/ml) to the culture during the 4-hr ^{51}Cr-release assay.

receptor for NK target antigens on NK-susceptible populations. This is an intriguing possibility in regard to the findings reported here, since CD2 can mediate cellular adherence as well as activation, and the antigen-specific cytotoxicity mediated by our NK lines appears to occur, in part, as a consequence of specific adherence. However, the CD2–LFA-3 adhesion pathway is thought to be antigen-independent (23), and peptide variability within the CD2 molecule has not been demonstrated. Furthermore, anti-Leu-5b (CD2) mAb did not substantially inhibit specific lysis in this study, nor did anti-LFA-3 (CD58) (Fig. 5). The possibility remains that epitopes on the CD2 antigen not recognized by this antibody may be involved.

The CD16 molecule (Fc_γ receptor) is capable of mediating antibody-dependent target lysis, and thus a role for CD16 in the specific cytolysis observed here must be considered. However, saturating concentrations of anti-Leu-11c mAb failed to inhibit cytotoxicity (Fig. 5), and $CD16^-$ $CD3^-$ clones also killed the relevant, stimulating EC line with specificity (data not shown); thus the surface expression of CD16 is not required for the observed phenomenon to occur.

Because it has been postulated that the induction of specialized endothelial vessels and lymphocyte homing play a role in allograft rejection (24) and because adhesion is critical to the interactions described above, we examined the ability of mAb 515, which recognizes a lymphocyte adhesion molecule thought to play a role in binding to high endothelial venules (8), to inhibit cytotoxicity. Although the effector cells uniformly expressed the molecule detected by mAb 515, the antibody had no effect on specific lysis.

Because EC-adherent lymphocytes, enriched in $CD16^-$ cells, appeared to preferentially lyse the ECs to which they initially bound (Fig. 1) and because the $CD16^+$ EC-reactive lymphoid lines mediated cytotoxicity in an allospecific fashion (Fig. 3), we also investigated whether these propagated lines adhered specifically to the relevant stimulating ECs. AL158 and AL178 were removed from their respective EC stimulators and tested for adherence in 20-min binding assays (Table 1). A shortened incubation was chosen due to the potent cytotoxic potential of the effector cells. EC180 served

Table 1. Adherence of NK lines to the relevant stimulating EC lines and irrelevant lines

Target cells	% adherent effectors	
	AL158	AL178
EC158	50.6 ± 5.1*	34.7 ± 3.9
EC178	39.5 ± 3.2	44.2 ± 2.6†
EC180	37.5 ± 3.6	33.6 ± 2.1

AL158 and AL178 were generated from the same individual and were stimulated with the EC lines EC158 and EC178, respectively, in the presence of rIL-2. Upon recovery after 3 weeks, the lymphoid cells were ^{51}Cr-labeled and utilized as effectors in a binding assay, with a 2:1 lymphocyte/EC ratio and a 20-min incubation. Values represent the percentage of labeled cells that remained adherent after the incubation and washes. The results are representative of four separate experiments.
*$P < 0.05$.
†$P < 0.01$.

as a mutually irrelevant target, to which AL158 and AL178 bound quite well (37.5% and 33.6%, respectively). This is not surprising due to the advanced state of lymphocyte activation, the known induction of nonspecific adhesion molecules such as the VLA antigens during activation (25), and the previously described enhanced adhesion of IL-2-activated lymphocytes to human ECs (17). AL158 and AL178 adhered to the two irrelevant EC lines to the same degree but demonstrated enhanced binding to the relevant stimulating EC lines (Table 1). Additional reciprocal binding experiments with EC-activated T-cell lines failed to demonstrate any specific binding, despite allospecific cytotoxicity (data not shown). These results suggest that the cytotoxic NK lines bind to their stimulating ECs via both nonspecific and specific ligand interactions, and further suggest that the basis for their specific cytotoxicity may lie, at least in part, with specific binding.

Taken together, these findings indicate the existence of CD3/Ti$^-$ antigen-specific killer cells, highly cytotoxic for microvascular ECs when propagated *in vitro* in the presence of ECs. Since these killer lines lack CD3-associated Ti gene rearrangements but express CD16 molecules and lyse NK-sensitive targets, we believe they are most appropriately designated NK cells. Although NK cells are not generally believed to mediate cytotoxicity in an antigen-specific manner, the results of experimental animal bone marrow grafts demonstrate that NK cells are the effectors of hybrid resistance to parental grafts (26), a phenomenon that appears to be genetically restricted and directed at the products of the noncodominant hematopoietic histocompatibility (Hh) genes (27), which are linked to the MHC complex in the mouse (28). Ciccone *et al.* (29) have described specific lysis of allogeneic cells after stimulation of CD3$^-$ CD2$^+$ CD7$^+$ lymphocytes in a mixed lymphocyte culture system. The "antigen-receptor" on these NK cells as well as those propagated in our study remains obscure. Previous attempts to absorb NK activity with target cells have suggested heterogeneity of both NK-recognition and NK-target structures (30–32). The EC target molecules of our NK lines do not appear to be MHC antigens. Other candidates include the "endothelial-monocyte antigens," proposed to be responsible for acute renal allograft rejection in HLA-identical grafts (33), and postulated MHC-linked antigenic systems equivalent to the Hh antigens in the mouse. Conceivably, the endothelium may express a set of polymorphic antigens, distinct from HLA, which serve both as adhesion molecules and targets of lysis for NK cells. Insofar as studies of organ allograft recipients in animals and humans have demonstrated early graft invasion by phenotypic and functional NK cells (34, 35) and documented that the presence of such cells is predictive of subsequent graft rejection (35, 36), it is hoped that further definition of NK cell–microvascular EC interactions will increase our understanding of rejection mechanisms. Finally, it is conceivable that NK cells may use a similar antigen-specific mechanism to recognize targets in addition to ECs. If so, it should be possible to generate a variety of target-specific NK lines in order to define the range of this antigen-receptor system.

We are grateful to Dennis Sasaki for assistance with cytofluorography, Leslie Tackett for excellent technical assistance, and Donna Jones and Linda Sheehan for assistance with preparation of the manuscript. We gratefully acknowledge Josan Chung and Nancy Berliner for their assistance with Southern blots. J.R.B. is the recipient of a Clinical Investigator Award (K08HL02126-01) from the National Institutes of Health. This work was supported in part by National Institutes of Health Grant HL13108 and a Research Advisory Group award from the Veterans Administration.

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