DENDRITIC CELLS THAT HAVE INTERACTED WITH ANTIGEN ARE TARGETS FOR NATURAL KILLER CELLS

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The term natural killer (NK)[1] cells describes a heterogeneous population capable of lysing a wide variety of tumor or virally infected cells (1–4). NK cells are relatively radioresistant and can be activated by a mixture of α and β interferons (Ifn) or inducers of Ifn such as polyinosinic polycytidilic acid (poly I:C). NK cells lyse target cells nonspecifically, i.e., without NK cells having had prior encounter with target cells. Although there is increasing evidence for a role of NK cells in host defense (5–13), the overall biological role of these cells is poorly understood.

We have previously shown (14) that NK cells, induced by poly I:C and cytotoxic for YAC-1 target cells, can regulate the antibody response in vitro by eliminating an accessory cell (AC)–antigen combination that is necessary for sustaining B lymphocyte proliferation. AC include a subpopulation referred to as dendritic cells (DC) that interact with antigen and are required for stimulation of both B and T lymphocyte proliferation (15–17). We report here that DC which have interacted with antigen are targets for NK cells and that NK cells can regulate T lymphocyte proliferation by this activity.

Materials and Methods

Mice. C3H/HeN, 5–6 wk old, were purchased from an SPF colony, Frederick Cancer Research Center Animal Production Area. BALB/c mice were also purchased from FCRI. Mice were housed in a laminar flow hood and given acidified water; food and bedding were not sterilized. Mice were 10–14 wk old when used.

Irradiation. Mice or cells were irradiated from a ^{137}Cs source at a rate of 200 rad/min.

Poly I:C and Interferons. Poly I:C was purchased from Sigma Chemical Co., St. Louis, MO. Poly I:C (No. P-1530; Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline and stored at −80°C. A purified mixture of lyophilized mouse α and β interferons (Ifn) (Lee Biomolecular Research Laboratories, Inc., San Diego, CA) was derived from cultures of mouse L929 cells stimulated with poly I:C; the preparation is free of poly I:C.

Antibodies and Complement. Anti-asialo GM1 antiserum (anti-AGM1) was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. A monoclonal anti-DC anti-

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[1] *Abbreviations used in this paper:* AMLR, autologous MLR; DC, dendritic cells; DTH, delayed-type hypersensitivity; E/T, effector/target; FCS, fetal calf serum; Ifn, a mixture of α and β interferons; Mϕ, macrophage; MLR, mixed lymphocyte reaction; NK, natural killer; NMS, normal mouse serum; poly I:C, polyinosinic polycytidilic acid; TdR, thymidine.

body, designated 33D1 (17), was a kind gift of Dr. Ralph M. Steinman, The Rockefeller University. Anti-Thy-1, a monoclonal rat antibody designated AT83A, was a generous gift of Dr. F. W. Fitch of the University of Chicago. Anti-Ly-1.1 and anti-Ly-2.1 monoclonal antibodies and Low-Tox-M rabbit complement (C′) were purchased from Accurate Chemical & Scientific Corp., Westbury, NY. Cells (10^7/ml medium, without fetal calf serum [FCS]) were treated with anti-Thy-1 (final dilution, 1:10), anti-Ly-1.1 (1:20), anti-Ly-2.1 (1:20), or anti-AGM1 (1:100), and C′ (1:10), for 1 h at 37°C; cells were washed twice after treatment.

Cell Lines. An autoreactive cloned T cell line derived from a CBA/N mouse ($Thy\text{-}1^+$, $Ly\text{-}1^-$, $Ly\text{-}2^-$, $L3T4^+$, and surface immunoglobulin $[sIg]^-$), designated clone L, was provided by Dr. J. Quintans, the University of Chicago. YAC-1, grown continuously and monitored regularly to be mycoplasma free, was derived from a Moloney virus–induced lymphoma in an A/Sn mouse and is widely used to assay the cytotoxicity of murine NK cells (18, 19).

Cell Preparations and Cultures. RPMI 1640 with 25 mM Hepes and L-glutamine supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 5×10^{-5} M 2-mercaptoethanol, and 5% FCS, and 50 U penicillin and 50 μg of streptomycin per 100 ml of medium, was used, except as noted in the text, for all cell preparations and the mixed lymphocyte reaction (MLR) and autologous MLR (AMLR) cultures. Medium without FCS was used to suspend cells during treatment with antibodies and C′.

Cell preparations were treated with carbonyl iron particles to remove most AC (including DC) by mixing 10^8 cells in 10 ml medium with 200 mg of carbonyl iron particles. The mixture was poured into a 100-mm petri dish (No. 3003; Falcon Labware, Oxnard, CA). After incubation for 1 h at 37°C (5% CO_2 in air, 100% humidity for these and all other incubations), the carbonyl iron particles were moved to one side of the dish with a magnet and the nonadherent cells were collected and washed twice with fresh medium. DC were removed from cell preparations by first treating cells with carbonyl iron particles to remove most AC, including DC, and then treating the cells with anti-DC antibody and C′ to remove remaining DC. 100 μl anti-DC antibody and 100 μl C′ were added per 800 μl, containing 10^7 cells to be treated; the cells were incubated for 1 h at 37°C and then washed twice in fresh medium.

DC were prepared by incubating 10^8 C3H fresh whole spleen cells suspended in 10 ml medium in 100-mm petri dishes (Falcon 3003) for 2 h; nonadherent cells were then removed by gentle pipetting and by washing plates three times with fresh medium to remove the vast majority of T and B lymphocytes. The adherent macrophages (Mϕ) and DC were incubated for an additional 22 h in 10 ml of fresh medium. The nonadherent cells removed after this incubation contain ~1–2% of all nucleated spleen cells present in the normal spleen and, for convenience, are referred to as DC.

For the one-way MLR, C3H spleen cells were treated with carbonyl iron particles and anti-DC antibody plus C′ to remove DC; these cells are referred to as responder cells. BALB/c spleen cells treated with carbonyl iron and anti-DC antibody plus C′ to eliminate DC were then irradiated with 2,000 rad; these cells are referred to as stimulator cells. 5×10^5 responder cells and 5×10^5 stimulator cells in 150 μl medium per culture were mixed with either 50 μl medium alone or medium containing a third and/or fourth cell population (DC and/or NK cells).

For the AMLR, the responder cells of the cloned T cell line were stimulated directly by $I\text{-}E^k$-bearing C3H DC; the responders proliferated rapidly so that many fewer cells were required and peak responses occurred earlier. Therefore, each culture contained 10^4 clone L cells either alone or mixed with DC and/or NK cells in a final volume of 200 μl.

In repeated titrations the number of DC required for optimal proliferation ($[^3H]$-thymidine ($[^3H]$TdR) incorporation) in both the MLR and AMLR was 3×10^4 DC per culture; 10^5 DC per culture usually caused <10% higher response and 10^4 DC per culture usually stimulated 30–40% lower response than did 3×10^4 DC per culture. Therefore, 3×10^4 DC per culture were used for all experiments.

MLR cultures were pulsed with 1 μCi [^{3}H]TdR in 50 μl medium, 24 h before harvesting on day 5 of culture. AMLR cultures were pulsed similarly on day 2 of culture and harvested on day 3. Uptake of [^{3}H]TdR by cultured cells was measured using a liquid scintillation system (LS-230; Beckman Instruments, Fullerton, CA).

Induction of NK In Vitro. Cultures were in flat-bottomed microwells (96-well plates, No. 3596; Costar, Data Packaging, Cambridge, MA). 10^7 cells per 200 μl of medium containing 1% FCS were induced with Ifn in 50 μl of the same medium. The cytotoxicity of cells harvested after 18 h incubation was assayed against ^{51}Cr-labeled YAC-1 target cells. Previous studies (20, 21) showed measurable induction as early as 4 h, peak activity at 18 h, and diminished activity by 24–48 h.

Assay of NK Activity. YAC-1 target cells (1×10^6) were labeled with 100 μCi ^{51}Cr during 1 h incubation at 37°C in 5% CO_2. Effector cells in 100 μl medium (10% FCS) were serially diluted in V-bottomed 96-well microtiter trays (Dynatech Laboratories, Inc., Alexandria, VA) and mixed with 5×10^3 ^{51}Cr-labeled YAC cells in 100 μl medium. Plates were spun at 650 *g* for 3 min. After 4 h at 37°C, 100 μl of each supernatant was measured for radioactivity with a gamma counter. The percent lysis was calculated using the formula: specific lysis = [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100. Spontaneous release was invariably <15% of the maximum release. Maximum release was calculated by mixing 50 μl ^{51}Cr-labeled target cells with 150 μl of a 1:20 dilution of lysing agent (No. CS-620-9; Fisher Scientific Co., Itasca, IL) and multiplying the count by 2. (Maximum release was 100% of the incorporated counts.) Experimental release was the average of duplicate cultures.

Controls for NK assays always included pooled spleen cells obtained from two normal mice 8–10 wk old and pooled spleen cells obtained from two similar mice given 100 μg poly I:C 24 h before sacrifice.

Results

Cytotoxicity and Lability of NK Cells. Spleen cells from normal C3H mice usually caused 20–30% lysis of labeled YAC-1 target cells at an effector/target (E/T) ratio of 100:1; spleen cells from mice injected with poly I:C 24 h before sacrifice usually caused 50–60% lysis at this ratio (20). The cytotoxicity of spleen cells from poly I:C–treated mice was increased additionally by irradiating donors (600 rad) 12 h before sacrifice to eliminate most conventional B and T lymphocytes. Such cells typically caused 60–70% lysis of labeled YAC-1 cells at an E/T ratio of 100:1. These preparations, however, contained AC including DC; treating the cells with carbonyl iron particles eliminated a majority of these cells and further enriched for cells cytotoxic for YAC-1.

The cells recovered after these procedures (poly I:C injection and x irradiation of donors and treatment of the cells with carbonyl iron particles) represented ~5% of all nucleated cells present in the normal spleen; the cells typically caused 70–90% lysis of labeled YAC-1 cells at an E/T ratio of 100:1 and >50% lysis at an E/T ratio of 12:1 (21). For convenience, these cells are referred to here as NK cells. Cytotoxicity of NK cells for YAC-1 was eliminated by treating the cells with anti-AGM1 and C′ but activity was not reduced by treating cells with anti-Thy-1, anti-Ly-1.1, or anti-Ly-2.1 and C′ (Fig. 1).

Cytotoxicity for YAC-1 or other target cells is usually measured in short-term (4–6 h) assays using cell densities and culture conditions comparable to those for the MLR. NK cells lost cytotoxicity within 24 h when cultured under these conditions (Fig. 2, *a* and *b*). NK activity in cultures can persist or be induced in vitro by Ifn, but this requires quite different culture conditions, namely 10-fold higher cell density (10^8 instead of 10^7 cells/ml) and fivefold lower FCS concen-

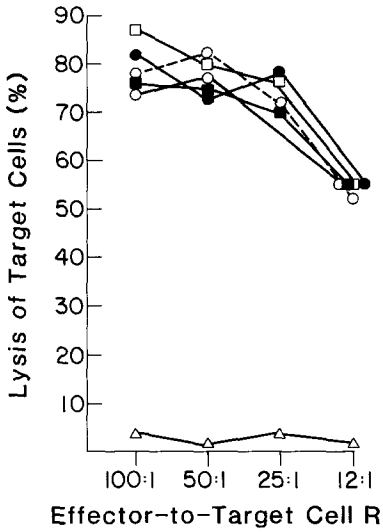

FIGURE 1. Cytotoxicity of NK cells. NK cells treated with anti-AGM1 and C′ (△) lost cytotoxicity for ^{51}Cr-labeled YAC-1 target cells. Cells in medium only (□), or treated with C′ only (■), anti-Thy-1 and C′ (○——○), anti-Ly-1.1 and C′ (○- - -○), or anti-Ly-2.1 and C′ (●) retain cytotoxic activity.

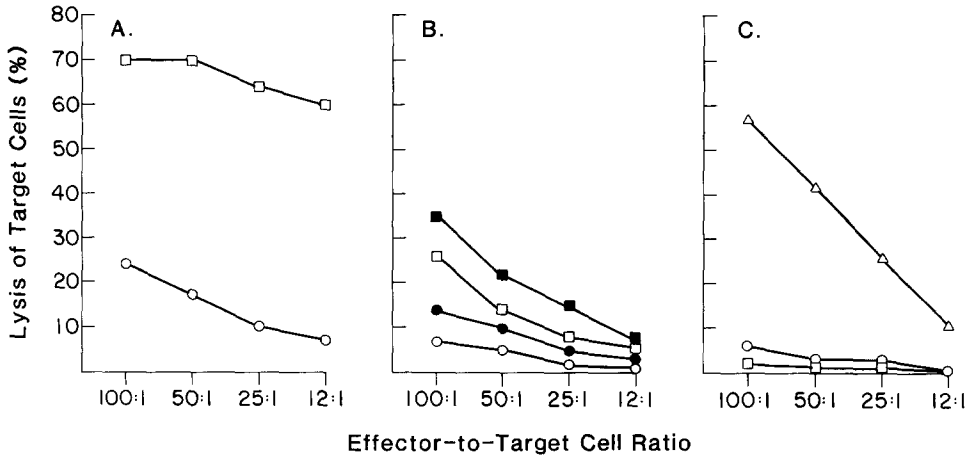

FIGURE 2. NK cells lose cytotoxicity irreversibly in culture. (*A*) NK cells (□) or fresh normal spleen cells (○) were assayed for cytotoxicity against ^{51}Cr-labeled YAC-1 target cells. (*B*) Aliquots of each preparation were cultured, 5×10^5 cells/culture, either alone or added to MLR cultures for 24 h. Cultures were harvested and assayed for cytotoxicity: NK cells alone (□), NK cells in MLR cultures (■), normal cells alone (○), and normal cells in MLR cultures (●). (*C*) NK cells (□) or normal cells (○) harvested after 24 h and recultured for 18 h, 10^7 cells/culture, in medium containing 1% FCS and 2,000 U Ifn were not cytotoxic; however, fresh normal spleen cells cultured under identical conditions with Ifn (△) were cytotoxic.

tration (1 instead of 5%). When NK cells were first incubated in the usual cultures for 24 h and then recultured at high cell density and low FCS concentration, NK activity was not restored or inducible by Ifn (Fig. 2*c*).

NK Cells Suppress or Eliminate DC. The loss of NK activity in cultures was

TABLE I
Suppression of the MLR Produced by NK Cells Is Reversed by DC

Day −1* NK cells	Day 0‡ DC	Day 5§ (cpm ± SD)
0	0	20,000 ± 419
+	0	4,902 ± 41
+	+	20,719 ± 1,469
0	+	29,884 ± 3,709

* Cultures on day −1 contained 5×10^5 responder, 5×10^5 stimulator, and 3×10^4 DC/culture. 5×10^5 NK cells per culture were added as indicated.
‡ 3×10^4 additional DC were added to cultures as indicated.
§ Cultures were pulsed with [^{3}H]TdR on day 4 and harvested on day 5. Data are mean cpm for three replicate cultures ± SD.

apparently irreversible. This meant that it might be possible to restore cultures by adding back the cell type eliminated or suppressed.

NK cells were added to cultures in varying numbers on the day cultures were set up; 5×10^5 NK cells per culture invariably caused >50% suppression of both the MLR and AMLR. $<10^5$ NK cells per culture caused little or no suppression while 10^6 NK cells per culture caused no more or only slightly more suppression than 5×10^5 NK cells per culture. Based on this finding, 5×10^5 NK cells per culture were used for all subsequent experiments.

Suppression caused by adding NK cells to cultures was reversed by adding an additional 3×10^4 DC/culture 24 h after NK cells (Table I); comparable results were obtained for the AMLR (data not shown). The findings confirmed the prediction that NK cells must produce their effect within 24 h, and suggested that DC were a likely target for NK cells. The following experiments tested this possibility directly by either culturing NK cells with DC for 24 h before adding responding lymphocytes to cultures, or culturing NK cells with responding lymphocytes for 24 h before adding DC to cultures.

The results were clear-cut for both the MLR and AMLR. DC incubated with NK cells for 24 h did not support responses (Table II), while lymphocytes incubated with NK cells for 24 h responded fully when DC were added to them (Table III). In addition, the experiments showed that NK cells treated with anti-AGM1 and C′ did not suppress and that 5×10^5 normal cells substituted for NK cells not only did not suppress but contained sufficient numbers of DC to sustain high responses in cultures without added DC. The essential findings in Tables II and III have been consistently observed in repeated experiments.

Antigen Is Required for DC to Become Targets for NK Cells. DC may have become targets for NK cells (see AMLR, Table II) because DC interacted with antigens in FCS medium. To test whether this was the case, we used medium containing 10% fresh normal C3H mouse serum (NMS medium), after having found that: (*a*) NK cells prepared and incubated with YAC-1 cells in NMS medium had cytotoxicity equal to NK cells prepared and incubated with YAC-1 cells in FCS medium; (*b*) NK cells lost cytotoxicity within 24 h equally in NMS or FCS medium; and (*c*) proliferative responses were low or absent in NMS medium. However, DC prepared in NMS medium and incubated in the medium

TABLE II

NK Cells Suppress or Eliminate DC Required for the AMLR and MLR

	Day −1*		Day 0‡	Day 5§
	DC	Additional cells	Responder cells	Response (cpm ± SD)
AMLR	+	None	+	114,170 ± 2,004
	+	NK	+	26,966 ± 2,987
	+	Normal cells	+	170,523 ± 4,495
	+	NK + anti-AGM1 + C′	+	173,068 ± 2,447
	−	None	+	720 ± 94
	−	NK	+	658 ± 113
	−	Normal cells	+	183,875 ± 4,130
	−	NK + anti-AGM1 + C′	+	1,995 ± 910
MLR	+	None	+	30,793 ± 879
	+	NK	+	7,840 ± 1,556
	+	Normal cells	+	67,410 ± 2,689
	+	NK + anti-AGM1 + C′	+	52,668 ± 7,350
	−	None	+	2,286 ± 163
	−	NK	+	3,978 ± 1,611
	−	Normal cells	+	30,143 ± 5,792
	−	NK + anti-AGM1 + C′	+	3,421 ± 906

* Cultures contained 3×10^4 DC as indicated and 5×10^5 NK or other additional cells as indicated. Cultures for the MLR also contained 5×10^5 stimulator cells.

‡ 10^4 responder cells were added to cultures for the AMLR and 5×10^5 responder cells were added to cultures for the MLR.

§ AMLR cultures were pulsed on day 2 and harvested on day 3; MLR cultures were pulsed on day 4 and harvested on day 5. Data are mean cpm for three replicate cultures ± SD.

for 24 h were fully active when washed and used in the usual MLR or AMLR cultures.

In a series of experiments, DC, NK cells, and antigen (sheep erythrocytes) were prepared in NMS medium. DC were incubated with or without antigen, and with or without NK cells. After 24 h, plates were centrifuged (650 *g* for 3 min), the medium carefully removed and replaced with FCS medium, and the washing procedure was repeated twice. Responder cells (with stimulator cells for the MLR) were added to cultures and antigen was added to those cultures that did not have antigen during the first 24 h. Cultures were pulsed and harvested as usual. The essential findings were invariable for both the AMLR and MLR: DC incubated with NK cells for 24 h in the absence of antigen supported high responses while DC incubated with antigen and NK cells for 24 h before the addition of responding cells did not support proliferation. The findings for a representative experiment using the AMLR are shown in Table IV.

Discussion

The term NK cells generally designates a broad category of heterogenous cells. Different interferons may induce different populations of NK cells and different populations of NK cells may be cytotoxic for different target cells. The cytotoxicity of our preparations was higher than that reported for NK lines and is as high (50% lysis of YAC-1 at E/T ratios of 5 to 10:1) as that reported for

TABLE III

Lymphocytes Are Not Targets for NK Cells

	Day −1		Day 0	Day 5
	Responder cells	Additional cells	DC	Response (cpm ± SD)
AMLR	+	None	+	74,403 ± 2,431
	+	NK	+	96,412 ± 4,476
	+	Normal cells	+	173,575 ± 11,403
	+	NK + anti-AGM1 + C′	+	154,768 ± 12,790
	+	None	−	1,317 ± 124
	+	NK	−	2,185 ± 43
	+	Normal cells	−	137,940 ± 4,828
	+	NK + anti-AGM1 + C′	−	2,436 ± 231
MLR	+	None	+	77,518 ± 11,548
	+	NK	+	81,370 ± 10,395
	+	Normal cells	+	100,356 ± 7,935
	+	NK + anti-AGM1 + C′	+	81,993 ± 6,923
	+	None	−	4,526 ± 1,541
	+	NK	−	24,053 ± 875
	+	Normal cells	−	81,346 ± 9,981
	+	NK + anti-AGM1 + C′	−	34,043 ± 1,451

Cell numbers and culture conditions were the same as in Table II; only the sequence of cell additions and incubation were varied as indicated.

TABLE IV

Only DC That Have Interacted with Antigen Are Targets for NK Cells in the AMLR

Day −1*			Day 0‡		Day 3§
DC	SRBC	NK	Responder cells	DC	Response (cpm ± SD)
+	0	+	+	0	8,780 ± 2,665
+	+	+	+	0	950 ± 145
+	+	+	+	+	13,522 ± 484
+	+	0	+	0	7,038 ± 362
+	0	+	+	+	17,038 ± 485
+	0	0	+	0	8,008 ± 1,118
0	0	0	+	+	20,998 ± 562
0	0	0	+	0	1,385 ± 147

* DC, NK cells, and sheep red blood cells were prepared in medium containing 10% fresh NMS. Cultures contained as indicated: 3×10^4 DC, 2×10^6 SRBC, and 5×10^5 NK cells in a final volume of 200 μl.

‡ Plates were centrifuged, 650 *g* for 3 min; medium was carefully removed and replaced with medium containing 5% FCS; the washing procedure was repeated twice. 10^4 responder cells and 3×10^4 DC suspended in FCS medium were added per culture as indicated. The final volume for all cultures was 200 μl.

§ Cultures were pulsed on day 2 and harvested on day 3. Data are mean cpm for three replicate cultures ± SD.

NK cells sorted by fluorescence-activated cell sorting (FACS) using a rat α murine Mϕ antibody (22). The present studies should make it possible to select lines of NK cells or obtain more homogeneous populations of NK cells that regulate DC function.

Since very few DC are required for lymphocyte proliferation, it is essential that DC be greatly depleted in cell preparations to demonstrate the critical requirement for these cells. We found that treating whole spleen cells with either carbonyl iron or with anti-DC antibody and C′ (at the cell densities and concentrations of reagents described here) is usually insufficient; i.e., the responder cells proliferated in the MLR without added DC. Dual treatment of cells, however, regularly eliminated responses unless DC were added. Preparations of NK cells are a potential source of contaminating DC and, in early experiments, we treated NK cells with anti-DC antibody plus C′ in addition to carbonyl iron particles. NK cells functioned as usual after dual treatment but, as shown in the present experiments, dual treatment of NK cells is not required to demonstrate the critical roles of DC and NK cells in cultures. In our hands, treatment of cells highly enriched for DC with anti-DC antibody and C′ always abolishes the capacity of the cells to function in the MLR and AMLR. The treatment caused only 50–60% of the cells in our preparations of DC to stain with trypan blue; however, the cells that did not stain also did not detectably contribute to the MLR or AMLR.

Even though our preparations of NK cells and DC are not homogeneous, we can estimate that $\sim 10^5$ NK cells were effective in eliminating the function of $\sim 10^4$ DC in culture. Considering the relatively static conditions in culture vs. the recirculation of cells in vivo, it appears that NK cells are present in sufficient numbers in vivo after activation (on the order of 5×10^6 NK cells per spleen) to be effective regulators of the $\sim 5 \times 10^5$ DC normally present in the spleen.

The activity of NK cells is short-lived in cultures but the total duration of increased NK activity after activation is short-lived in vivo as well. For example, NK activity was elevated above background levels for only 18–36 h after injection of poly I:C or Ifn; also, intravenous antigen causes a similarly brief elevation of NK activity 2–4 d after immunization (21), presumably caused by Ifn and/or IL-2 released from responding lymphocytes (23–28). Thus, a single "pulse" of inducers of NK activity or immunization provides only a brief interval during which DC/antigen function may be monitored by increased NK activity.

It is important to emphasize that, in the present experiments, NK cells, DC, and responding lymphocytes in the MLR were all syngeneic; thus, the usual relationship between these cell types in vivo was maintained in vitro. We have not determined yet whether NK cells bind to and lyse syngeneic DC that have interacted with antigen but the effect of the interaction is profound and apparently irreversible, since DC function did not return after NK activity was lost. Unfortunately, spontaneous release of ^{51}Cr from labeled DC is too high to use this method to directly measure NK cytotoxicity for DC. While NK cells may release soluble mediators that cause cytoxicity (29–31), such factors would have to be released in a diffusion-limited space to produce the effective measured responses, since we could restore responses by adding DC back to cultures. As would be expected from this finding, we found repeatedly that medium from

NK cells cultured alone or in MLR cultures supported normal MLR and AMLR cultures.

Spontaneous release of ^{51}Cr from labeled splenic lymphocytes was too high to determine whether NK cells are indiscriminately cytotoxic for these cells. However, 5×10^5 NK cells incubated with 10^4 cloned lymphocytes in the AMLR did not suppress at all the proliferative response when DC were added 24 h later (Table III), indicating that cells of the lymphocyte line are certainly not targets for NK cells. Furthermore, spontaneous release of ^{51}Cr from labeled cloned lymphocytes (clone L used for the AMLR) was low; such cells used as targets for NK cells were not lysed. Conceivably, after stimulation by DC and antigen, lymphocytes became targets for NK cells, but all of the observed effects caused by NK cells could be accounted for by elimination or suppression of DC that had interacted with antigen.

While Mϕ are not required for the MLR, small numbers of Mϕ increase responses up to twofold (P. Shah, unpublished results), similar to their effect on the antibody response (15). Mϕ are avidly phagocytic, express high levels of surface Ia, and can present antigen to sensitized T lymphocytes (32). However, we have thus far been unable to demonstrate that NK cells have any consistent effect when mixed with Mϕ and antigen for 24 h before adding DC and responding lymphocytes to cultures.

Presumably, the interaction of DC with antigen results in the expression of surface structures that are a target for NK; i.e., by combining with antigen, DC become altered-self cells comparable to virally infected or malignantly transformed cells considered the usual targets for NK cells. The capacity of NK cells to kill these target cells is independent of the expression of specific tumor or viral antigens. By analogy, DC that have interacted with antigen may also express molecules unrelated to that antigen. The target molecules might be previously unexpressed or unexposed differentiation markers, H2 or H2-like molecules, or possibly the receptor for transferrin (33, 34). If the target molecules are ubiquitous, like the receptor for transferrin, the interaction of DC with antigen may cause expression of a much higher density of target molecules, converting DC (or other cells) from non-targets or poor targets or vulnerable targets. It is possible to think of the NK system as providing a general mechanism through which an individual can eliminate those of his own cells that become "not self." By limiting antigen presentation by DC, NK would provide a common mechanism for regulating B and T lymphocyte proliferation. Indeed, in experiments to be reported separately, we found that NK cells suppressed the IgM antibody response in vitro by the same mechanism, the elimination or suppression of DC that had interacted with antigen.

T cells that proliferate in the MLR include Ly-1^+2^- T cells which mediate delayed-type hypersensitivity (DTH) (35–37). Others have reported that Tilorone, an inducer of Ifn (38), or Ifn itself (39), given at the time of antigenic challenge to sensitized mice, markedly suppressed DTH. We found similar results with poly I:C. In experiments to be reported separately, lymphocytes obtained from C3H mice sensitized to BALB/c lymphocytes 8 d previously were injected with alloantigen into footpads of irradiated (600 rad) C3H recipients. The local DTH reactions were eliminated if DC were removed from the transferred cells

or if NK cells were added to the cell mixture; however, responses were restored by adding DC to these mixtures. Thus, NK cells may regulate some T cell responses in vivo as well as in vitro by monitoring DC/antigen presentation.

Summary

Natural killer (NK) cells (poly I:C induced, x-ray resistant, nonadherent, Thy-1$^-$, Ly-1.1$^-$, Ly-2.1$^-$, anti-asialo GM1–positive, and cytotoxic for YAC-1) suppressed T lymphocyte proliferation in mixed lymphocyte reaction (MLR) and autologous MLR cultures. Dendritic cells (DC) were required for proliferation of lymphocytes in both responses. The question whether lymphocytes or DC were the targets for NK cells was resolved by taking advantage of the fact that NK cells, but not DC, lose activity after 24 h in culture. Three findings indicate that DC, not lymphocytes, are targets for NK cells. First, responses suppressed by NK cells were fully restored by adding small numbers of DC to cultures 24 h after NK cells had been added. Second, DC incubated alone with NK cells and antigen for 24 h did not stimulate proliferation of lymphocytes. Third, lymphocytes incubated alone with NK cells for 24 h proliferated normally when DC were added. Additional experiments showed that DC became targets only after interaction with antigen. Thus, we suggest that NK cells may regulate lymphocyte proliferation by monitoring antigen presentation by DC.

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