Brief Definitive Report

A CLONED CELL LINE MEDIATING NATURAL KILLER CELL FUNCTION INHIBITS IMMUNOGLOBULIN SECRETION*

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We have previously shown that T cell clones can mediate multiple biologic activities (1). Inducer T cell clones, for example, may stimulate immunoglobulin (Ig) secretion and activate mast cell and T cell proliferation (1, 2). Similarly, we found (3) that clones with natural killer cell activity can also mediate antibody-dependent cellular cytotoxicity. Because these clones lysed syngeneic B lymphocytes, we considered the possibility that this cell might be involved in the regulation of Ig secretion. We test this hypothesis in this report. We show that the antibody responses of T plus B lymphocytes containing <1% cloned natural killer (NK) cells are severely impaired, and the primary cellular target of suppression is the B cell. We suggest that some NK cells may regulate Ig secretion through the recognition of marker molecules expressed on the surface of activated B lymphocytes not linked to the major histocompatibility complex.

Materials and Methods

Animals. Cells for cloning were obtained from C57BL/6 mice. Cells from C57BL/6 or BALB/c mice (obtained from The Jackson Laboratory, Bar Harbor, ME) were used to produce conditioned medium.

Antisera. Lyt-1.2 and Lyt-2.2 antisera, prepared as described (4), were kindly donated by Dr. F. W. Shen; NK-1 antisera were donated by Dr. G. Koo; monoclonal (mc) anti-Thy-1.2 were donated by Dr. Ed Clark; and mc-anti-Lyt-1 and mc-anti-Lyt-2 were donated by Dr. J. Ledbetter and Dr. L. Herzenberg.

Cell Culture and Cloning. Culture conditions for initiation and maintenance of cell lines have been described (5).

Frozen Cells for Storage. Cloned cells were frozen in liquid N_2 and thawed with complete recovery of growth and function, using a modification (5) of the method of Lionetti and colleagues (6).

Assays of Biologic Function. Lysis of the YAC-1 lymphoma, an NK-sensitive tumor (7, 8), was used to define NK activity, as previously described (3). Preparation of selected lymphoid populations (1), lipopolysaccharide (LPS)-activated B cells (3), and stimulation and enumeration of plaque-forming cells (PFC) (9) are detailed elsewhere.

Cellular Reconstitution of Irradiated Adoptive Hosts. Highly purified B cells (10^6), prepared as above, were injected into a lateral tail vein of irradiated (700 rad) recipients with 10 μg trinitrophenyl (TNP)-Ficoll (TNP:Ficoll=24:1). Increasing numbers of Ly-1^-2^-NK-1^+/11 clones (Cl.) were injected separately into a second lateral tail vein. PFC responses were tested 5 days later using TNP-coupled sheep erythrocytes (SRBC) as indicator cells (9). Each group represents the mean of three to five mice.

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To test T cell function in vivo, 10^6 T cells were enriched as previously described (3) and injected separately from Cl.Ly-1$^-$2$^-$NK-1$^+$/11 as above. Spleen cells were removed 7 d later and incubated with purified B cells and SRBC in vitro. Anti-SRBC PFC were enumerated 4 d later. Each group represents the mean of three individual cultures. In no case was the standard error of the mean >15%.

Results

Cl.Ly-1$^-$2$^-$NK-1$^+$/11 Lyses Activated B Lymphocytes. We previously analyzed cellular targets of cloned NK-like cells. Susceptible targets include LPS-activated B lymphocytes, but concanavalin A (Con A)-activated T cells, thymocytes, fetal liver, bone marrow cells or mast cell clones are relatively resistant to lysis (Fig. 1).

Cl.Ly-1$^-$2$^-$NK-1$^+$/11 Inhibits Ig Secretion In Vitro. Because lysis of B cell targets occurs very efficiently, with effector-to-target ratios as low as 1:10, we tested the ability of NK-1$^+$/11 cells to inhibit Ig secretion by B cells. Addition of small numbers of NK-1$^+$/11 cells to spleen cells caused potent inhibition of Ig secretion. This suppression was nonspecific, inhibiting primary responses both to sheep or chicken erythrocytes (Table IA) or total Ig secretion (data not shown). Suppression was observed when cloned cells constituted as few as 0.2% of total cells in culture. Cell-free supernatant from this clone did not reduce Ig secretion, suggesting that this inhibition was mediated directly by cells. Because NK-1$^+$/11 does not lyse or phagocytize erythrocytes, spleen cells rather than antigen appeared to be the likely targets of suppression. In addition, because the doubling time of NK-1$^+$/11 was ~24 h, this suppression was probably not caused by crowding in cell cultures. Other T cell clones with similar growth rates do not inhibit Ig secretion (5). To test whether this suppression might result from direct action on B lymphocytes, T cell-replacing factor (10) was added to cultures containing either B lymphocytes or B cells and increasing numbers of NK-1$^+$/11 cells. As few as 5×10^3 NK-1$^+$/11 cells inhibited Ig secretion by 10^6 B cells (Table IB).

Cl.Ly-1$^-$2$^-$NK-1$^+$/11 Inhibits Ig Secretion In Vivo. To determine whether Cl.Ly-1$^-$2$^-$NK-1$^+$/11 cells could mediate suppression under physiologic conditions, these cells were injected by vein into irradiated recipient mice reconstituted with selected lymphocyte populations. Increasing numbers of NK-1$^+$/11 cells were injected separately into a different tail vein from test lymphocytes to eliminate the possibility of an in vitro interaction. Irradiated recipients were infused with 10^6 B cells, challenged

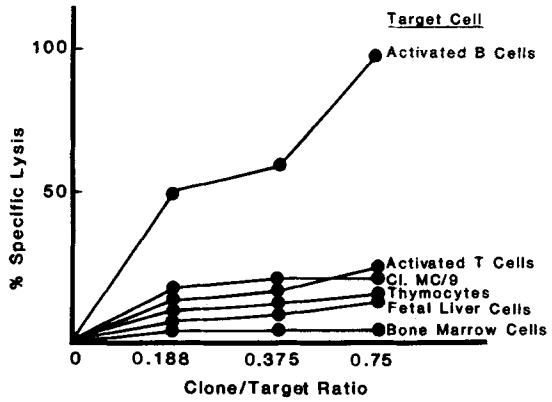

FIG. 1. Cellular targets of Cl.Ly-1$^-$2$^-$NK-1$^+$/11. Cl.Ly-1$^-$2$^-$NK-1$^+$/11 incubated with radiolabeled cells from normal tissues for 3.5 h (see Materials and Methods).

TABLE I

Inhibition of Ig Secretion by Cl.Ly-1$^-$2$^-$NK-1$^+$/11

	Number of Ly-1 cells per culture ($\times 10^5$)	Cloned cells per culture ($\times 10^4$)	Final concentration of T cell-replacing factor/culture	Anti-SRBC Ig PFC/culture	Anti-CRBC Ig PFC/culture
			%		
A	0	0		190 ± 21	190 ± 6
	2	0		2,000 ± 345	840 ± 43
	2	1		250 ± 29	33 ± 4
	2	0.2		550 ± 22	550 ± 22
B	0	0	0	34 ± 4	ND
	0	0	10	668 ± 52	ND
	0	5	10	40 ± 5	ND
	0	1	10	180 ± 85	ND
	0	0.5	10	312 ± 40	ND

(*A*) Cl.Ly-1$^-$2$^-$NK$^-$1$^+$/11 cells were added to mixtures of Ly-1 cells from B6 mice immune to sheep or chicken erythrocytes (CRBC), 10^6 nonimmune B6 B cells, and 10^6 erythrocytes. Cell cultures were incubated for 4 d before anti-SRBC or CRBC PFC were enumerated, as described in Materials and Methods. (*B*) Cl.Ly-1$^-$2$^-$NK$^-$1$^+$/11 cells were added to 10^6 B cells, 10^6 erythrocytes, and T cell-replacing factor before enumeration of anti-sheep erythrocyte PFC 4 d later (see Materials and Methods).

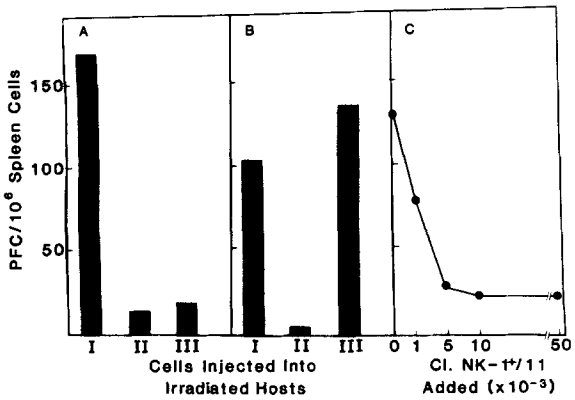

FIG. 2. Inhibition of Ig secretion in vivo. (A) Irradiated recipient mice (700 rad) were injected with 10 μg TNP-Ficoll and (I) 10^6 B cells, (II) no cells (sham injection), or (III) 10^6 B cells plus 10^5 Cl.Ly-1$^-$2$^-$NK-1$^+$/11 (see Materials and Methods). Anti-TNP PFC were determined 7 d later. (B) Irradiated recipient mice (700 rad) were injected with (I) 10^6 T cells, (II) no cells (sham injection), or (III) 10^6 T cells + 10^5 Cl.Ly-1$^-$2$^-$NK-1$^+$/11. Spleen cells were removed 7 d later, and 10^5 viable cells were incubated with 10^6 B cells plus sheep erythrocytes in vitro. Anti-SRBC PFC were enumerated 4 d later (see Materials and Methods). (C) Dose-response of inhibition of B cell Ig secretion in vivo by Cl.Ly-1$^-$2$^-$NK-1$^+$/11. Irradiated recipient mice (700 rad) were injected with 10 μg TNP Ficoll, 10^6 B cells, and increasing numbers of Cl.Ly-1$^-$2$^-$NK-1$^+$/11. Anti-TNP PFC were determined 7 d later.

with TNP-Ficoll, and tested for anti-TNP PFC 7 d later (Fig. 2A). The anti-TNP response of mice infused with as few as 5×10^3 NK-1$^+$/11 cells (0.5% of the total nonirradiated cells in the host) was virtually eliminated. Similar to in vitro experiments, suppression was observed when NK-1$^+$/11 represented as little as 0.1% of the total lymphocytes (Fig. 2C). To test the in vivo effect of NK-1$^+$/11 cells on the development of T helper function, irradiated mice were infused with Ly-1 cells and SRBC for 5 d alone, with graded numbers of NK cells. There was no inhibition of T helper activity by NK cells tested in vitro, as described in Materials and Methods, except when Ly-1 cells were incubated with very large numbers of NK1$^+$/11 (1:1 NK-Ly-1 ratio).

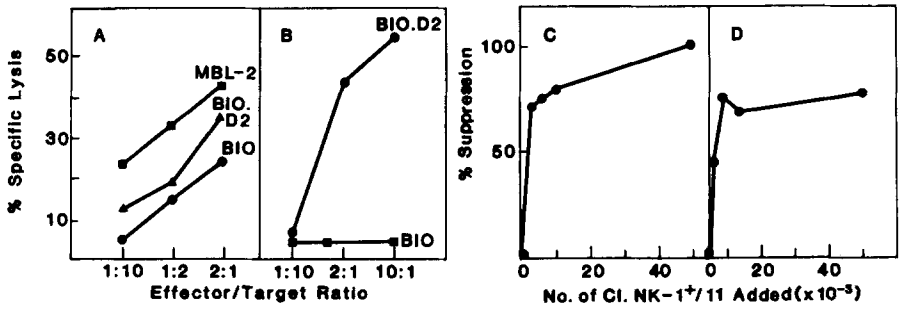

FIG. 3. Lysis and inhibition of Ig secretion in MHC-different target cells. (A) Cl.Ly-1$^-$2$^-$NK-1$^+$/11 cells were incubated with radiolabeled MBL-2 (C57Bl/6 lymphoma), B10.D2N LPS-activated B cells, or B10 LPS-activated B cells for 3.5 h, and lytic activity was determined from release of ^{51}Cr. (B) Lysis of radiolabeled B10.D2N or B10 LPS-activated B lymphocytes by cells from line 18, a B10 T cell growth factor-dependent CTL line stimulated for 3 min in vitro with B10.D2 cells. (C) Inhibition of Ig secretion in syngeneic B6 spleen cells. Increasing numbers of Cl.Ly-1$^-$2$^-$NK-1$^+$/11 were incubated with 10^6 B6 spleen cells + 10^6 sheep erythrocytes in vitro (see Materials and Methods). Anti-SRBC Ig secretion was determined 4 d later. Control cultures containing spleen cells without SRBC yielded 20 PFC/culture, and spleen cells in the absence of NK clone produced 132 PFC/culture. (D) Inhibition of Ig secretion in H-2-congenic B10.D2N spleen cells. Spleen cells in the absence of NK clone produced 128 PFC/culture, whereas control cultures without antigen yielded 40 PFC/culture.

Cl.Ly-1$^-$2$^-$NK-1$^+$/11 Lyses Activates B Cells in a Non-H-2-restricted Fashion. Because activated B lymphocytes express MHC determinants (11, 12) and these can play an important role in T cell suppression (13, 14), we tested whether NK-1$^+$/11 cells recognized MHC-linked determinants as judged by lysis. In contrast to H-2-specific cytotoxic T lymphocyte (CTL) lines, NK-1$^+$/11 cells lysed B lymphocytes from mice congenic at the H-2 locus (Fig. 3 A and B). NK-1$^+$/11 cells lyse activated B lymphocytes from all mouse strains tested with equal efficiency, including strains congenic at the GIX, H-2, Ly-1, Ly-2, and Thy-1 loci (not shown).

Inhibition of B Cell Ig Secretion by Cl.Ly-1$^-$2$^-$NK-1$^+$/11 Is Not Restricted to MHC-identical Target Cells. To determine whether inhibition of B cell Ig secretion by NK-1$^+$/11 was restricted by MHC genes, NK-1$^+$/11 cells were incubated with SRBC and spleen cells from B6 or B10.D2N mice. Anti-SRBC PFC activity was almost completely abolished in cell cultures containing as few as 10^3 NK-1$^+$/11 cells (0.1% of total cells), regardless of H-2 haplotype (Fig. 3C and 3D). Similarly, 83% inhibition of the maximum PFC response of BALB/c spleen cells was noted in cultures containing 5 $\times$ 10^3 NK-1$^+$/11 cells. Cultures of enriched B cells contained ~50% viable cells after incubation with or without NK-1$^+$/11.

Discussion

NK cells display at least two patterns of lytic activity in mice (15). One pattern includes lysis of virus-infected tumor targets such as the YAC-1, RL♂, MBL-2, or RBL-5 lymphomas by spleen cells. A second often follows activation by substances such as Calmette-Guerin bacillus, interferon inducers, certain tumors, or other undefined factors, and is characterized by acquisition of lytic activity of additional tumors (e.g., EL4 lymphoma or P815 mastocytoma) (3, 15). These latter cells have been termed "activated" NK cells. We previously described (3) a cloned cell line, Cl.Ly-1$^-$2$^-$NK-1$^+$/11, that mediates activated NK cell function. We found that this clone also lysed a variety of nonmalignant lymphoid cells, including B but not T lympho-

cytes. We therefore postulated that it might play a role in the regulation of Ig secretion.

In this report, we show that Cl.Ly-1$^-$2$^-$NK-1$^+$/11 cells potently inhibit Ig secretion by B cells both in vitro and in vivo. The latter finding suggests that this mechanism may play a role in the regulation of normal antibody response. Indeed, Ly-1$^-$2$^-$ spleen cells contain potent Ly-1$^-$2$^-$5$^+$ cells (L. Glimcher, L. Boudreau, and H. Cantor, manuscript in preparation) that directly inhibit Ig secretion by B cells, as measured by a reverse PFC assay.

Our data suggest that activated B cells rather than antigen-presenting cells are the cellular target for Cl.Ly-1$^-$2$^-$NK-1$^+$/11 because, in our hands, adherent cells are not required to support Ig secretion in cell cultures supplemented by the TRF preparations (in contrast to supplementation by T helper cells) (data not shown). However, we cannot exclude the possibility that in some cases macrophages may represent an additional target cell. Although we occasionally observed that large numbers of NK-1$^+$/11 cells suppress T helper cells educated in vivo (tested by subsequent helper activity measured in vitro), in most instances, this is caused by residual NK-1$^+$/11 acting directly on B cells during the in vitro assay and can be eliminated by treating the educated cells with αNK-1.1 + C′.

To define the gene controlling the target structures on activated B cells, we tested a variety of LPS-activated B cells from inbred mice congenic at different loci. Cl.Ly-1$^-$2$^-$NK-1$^+$/11 lysed B cells from all strains tested, including strains that differed at the MHC, Lyt, Thy-1, and GIX loci. Because target B cells were often grown in media supplemented with FCS, we considered the possibility that a serum constituent might be recognized on activated B cells. However, such recognition probably does not account for target cell sensitivity to lysis because normal mouse mast cells, thymocytes, and certain tumors that are also grown in FCS-supplemented cultures are not susceptible to lysis. Moreover, this cannot explain NK suppression of B cells in vivo because the adoptively transferred lymphocytes were never exposed to FCS.

LPS-activated B lymphocytes express viral glycoproteins. The possibility that the target molecules may cross-react with viral determinants similar to those found on tumor targets is consistent with our observation that the YAC-1 lymphoma inhibits lysis of activated B cell targets by Cl.Ly-1$^-$2$^-$NK-1$^+$/11 (data not shown). However, so far we have been unable to block lysis of B cells using anti-gp 70 antibody or other anti-viral antisera reagents.

The observation that Ig secretion can be inhibited by Cl.Ly-1$^-$2$^-$NK-1$^+$/11 suggests a novel mechanism for an immunoregulation circuit in which control of Ig secretion is mediated by direct recognition of a B cell surface structure, independent of antigen. This cell surface marker on activated B cells may be recognized by NK-like regulatory cells and serve to "mark" particular cells for death after activation by either T cell products, certain viruses (16), or a given foreign antigen. In the latter case, this might result in deletion of B cell clones bearing Ig receptors specific for that antigen. The availability of continuously propagatable B cell lines should allow direct examination of this hypothesis and further definition of B cell surface molecules recognized by activated NK-like clones.

Summary

We previously described a cloned cell line that combines information for a unique display of cell surface antigens and specialized function similar to activated natural

killer (NK) cells. In addition to conventional cellular targets such as the YAC-1 and MBL-2 lymphomas, this cloned line also lysed lipopolysaccharide-activated B lymphocytes. To determine whether some NK cells can inhibit B cell function, we tested the ability of NK-like clones to suppress Ig secretion in vitro and in vivo. These cloned cells suppressed Ig secretion when they constituted as few as 0.2% of the total cell population and inhibition did not require identity at the H-2 locus. We suggest that some NK cells might recognize non-major histocompatibility complex gene products on activated B lymphocytes and lyse these cells, and this might represent a fundamental cell-cell interaction that regulates antibody secretion by activated B cells.

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