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Activation of Natural Killer Function Through the T11/E Rosette Receptor *

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Natural killer (NK) cells have been identified as a population of circulating lymphocytes capable of mediating direct cytotoxicity against a variety of target cells without prior immunization. There is now considerable experimental evidence to suggest that this lymphocyte subpopulation is capable of immune surveillance against tumor cells in vivo and that NK cells may exert important regulatory functions within the immune system [1, 2, 6, 24, 25]. In recent years it has become evident that NK cells themselves can be regulated through interaction with various lymphokines such as interferon and interleukin 2 (IL-2) [5, 7, 13, 22, 26, 27]. In the present studies, we demonstrate that the cytotoxic function of NK cells can be markedly enhanced through activation of the receptor for sheep erythrocytes (T11/E rosette receptor) and that this enhancement is comparable to that observed following IL-2 activation.

The T11/E rosette receptor antigen has been shown to be a pathway for antigen-independent activation of peripheral blood T lymphocytes [12] and is expressed on thymocytes, T cells and NK cells [3, 9, 14]. Three different epitopes on the T11 antigen have been defined. Activation through the T11 complex is independent of expression of T-cell receptor for antigen and has been found to occur with immature thymocytes as well as with T3 negative NK clones [18]. In addition, it has been demonstrated that cellular activation with anti-$T11_2$ and anti-$T11_3$ monoclonal antibodies induces MHC-independent killing by cytolytic T-cell clones as well as cytotoxicity of NK clones against otherwise resistant targets [23].

In the present studies, we examined whether the cytotoxicity of purified peripheral blood NK cells could be activated via the T11/E rosette receptor and compared these effects to those observed following IL-2 activation. Human NK cells were purified from peripheral blood by immunofluorescent flow cytometric cell sorting of NKH1 positive cells [8, 17]. NKH1 has previously been demonstrated to be a pan-NK cell antigen expressed by cells that morphologically appear as large granular lymphocytes and which comprise approximately 12% of peripheral blood mononuclear cells (PBMC). All cells in peripheral blood with NK activity express $NKH1^+$ but this antigen is not expressed by T cells, B cells, monocytes or granulocytes [8, 17]. When PBMC are separated into $NKH1^+$ and $NKH1^-$ fractions (Fig. 1 A) all of the natural cytotoxicity against a standard NK target cell, K562, is contained within the $NKH1^+$ population. Following 18 h incubation with recombinant IL-2 (Fig. 1 B), the NK activity of unseparated PBMC is significantly enhanced, but cytotoxicity remains confined to the $NKH1^+$ subset. Results shown in Fig. 1 C demonstrate that enhancement of cytotoxicity can also be seen follow-

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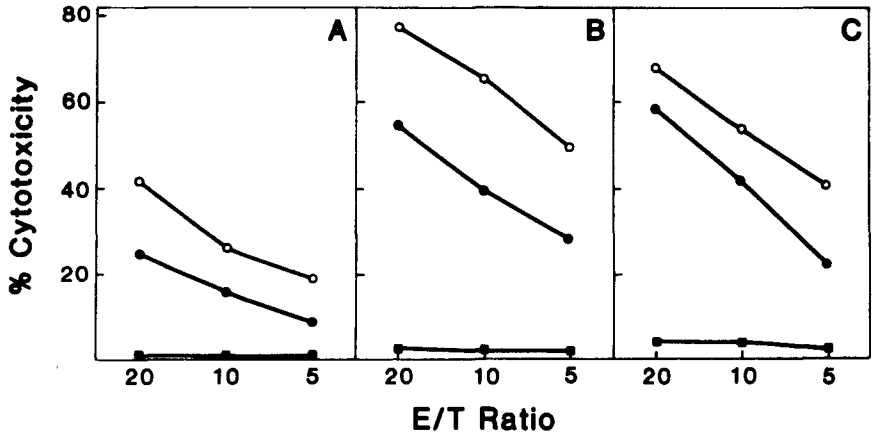

Fig. 1. NK activity of PBMC (●–●), NKH1$^+$ (○–○), and NKH1$^-$ (□–□) cells were measured against K562 target cells after incubation with either media (*panel A*), purified recombinant *IL-2* (*rIL-2*) (*panel B*), or T11$_{2/3}$ monoclonal antibodies (*panel C*). Cytotoxicity assays were performed according to a standard chromium release method previously described [8, 19]. All experiments were done in triplicate using V bottom microtiter plates. Medium was RPMI 1640 + 5% pooled human AB serum and 1% penicillin streptomycin. Assays were performed at various effector/target (E/T) ratios using between 3000 and 5000 K562 target cells/well

ing 18 h incubation with anti-T11$_{2/3}$ monoclonal antibodies. Moreover, the triggering of peripheral blood NK cells with anti-T11$_{2/3}$ antibodies is as effective as with rIL-2 and is also restricted to NKH1$^+$ cells.

To directly examine the effect of anti-T11 monoclonal antibodies on effector-target cell binding, we evaluated the ability of activated effector cells to form cell conjugates with K562 targets. As shown in Table 1, preincubation of NKH1$^+$ cells with either anti-T11$_{2/3}$ or IL-2 significantly enhances conjugate formation. This effect is stronger for anti-T11$_{2/3}$ than for IL-2 triggering. Interestingly, conjugate formation is also induced in NKH1$^-$ cells when activated through the T11 pathway, although these cells are not able to mediate direct cytotoxicity. In contrast, IL-2 does not induce the formation of conjugates in the NKH1$^-$ population. Blocking studies using anti-LFA-1 antibody (data not shown) suggest that the T11-induced enhancement of cytotoxicity is at least in part due to an increased binding of effectors to target cells that is mediated through LFA-1 antigen [23].

To test whether T11 activation of NKH1$^+$ cells would induce cytotoxicity

Table 1. Effector: target cell conjugates induced by anti-T11$_{2/3}$ and recombinant IL-2

Time[a]	NKH1$^+$ cells			NKH1$^-$ cells		
	Media	Anti-T11$_{2/3}$	rIL-2	Media	Anti-T11$_{2/3}$	rIL-2
20′	10[b]	50	44	4	88	0
60′	10	68	52	8	94	8
120′	32	72	46	6	96	2

[a] Following 18 h incubation, effector cells were incubated with K562 target cells (20/1; E/T ratio) for 20, 60, or 120 min at 37° C prior to enumeration of conjugates.

[b] Each value represents percent K562 target cells forming conjugates of at least one effector cell. Peripheral blood mononuclear cells were separated into NKH1$^+$ and NKH1$^-$ populations by immunofluorescent cell sorting and subsequently incubated with either media, anti-T11$_2$ and anti-T11$_3$ monoclonal antibodies (1:250 final ascites dilution) or recombinant IL-2 (1000 U/ml) for 18 h at 37° C.

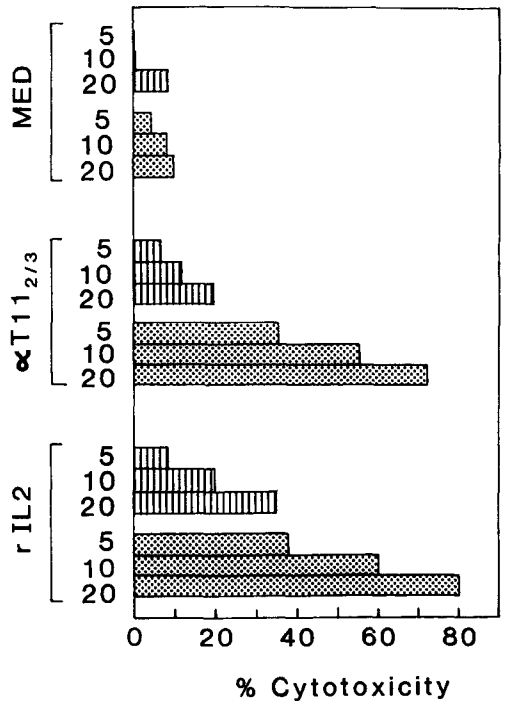

Fig. 2. Cytotoxic activity of PBMC (▨) and purified NKH1$^+$ (⁙) cells against the NK-resistant Burkitt lymphoma line Daudi. Assays were performed after 18 h incubation with media, $T11_{2/3}$ antibodies, or *rIL-2*. Methods for purification of NKH1$^+$ cells and cytotoxicity assays are as described for Fig. 1

against "NK-resistant" tumor cells, we performed similar experiments using Daudi Burkitt's lymphoma cells as targets. As shown in Fig. 2, PBMC and resting NKH1$^+$ cells exhibit very little killing activity against Daudi cells. However, when purified NKH1$^+$ cells were stimulated with $T11_{2/3}$ antibodies, significant cytotoxicity against Daudi was induced. Similar results were observed after activation with purified recombinant IL-2 and with both methods of activation, this enhancement was found only within the NKH1$^+$ population. T11-induced enhancement of cytotoxicity was also found against other tumor lines (data not shown).

In summary, this study demonstrates that the cytotoxicity of purified NKH1$^+$ peripheral blood NK cells can be significantly enhanced via the T11/E rosette receptor pathway. The extent of NK enhancement after an 18-h incubation period is quite similar to the effects seen following IL-2 activation. Moreover, our data indicate that activation using either IL-2 or monoclonal $T11_{2/3}$ antibodies induces cytotoxicity against so-called NK-resistant target cells. The cell separation studies done in conjunction with in vitro activation consistently demonstrate, that cytotoxicity is contained exclusively within the NKH1$^+$ cell fraction.

Although IL-2 and T11 activation lead to similar degrees of enhancement of cytotoxicity, there are differences in the effects of IL-2 and anti-T11 which suggest that distinct cellular mechanisms may be involved. One major difference is the enhancement of conjugate formation in the NKH1$^-$ population that can be induced by anti-T11 but not by IL-2 (Table 1). It is also known that 1 h incubation with IL-2 is sufficient for enhancement of cytotoxicity [15], whereas the effects of anti-T11 antibodies require at least 6–8 h stimulation before significant enhancement can be detected (data not shown). The effects of these two activators on cloned NK and CTL effectors have also shown disparate results since only anti-T11 is able to induce killing of resistant targets by cultured cell lines [8, 17, 19]. In addition, it has been observed that IL-2 induces proliferation as well as cytotoxicity of NKH1$^+$ purified NK cells, whereas anti-$T11_{2/3}$ antibodies do not induce in vitro proliferation of these cells (data not shown).

Since it is known that $T11_{2/3}$ activation results in rapid expression of IL-2 receptor, it is possible that IL-2 may also play a significant role in the functional effects seen following stimulation with anti-$T11_{2/3}$. In this regard, the addition of both IL-2 and anti-$T11_{2/3}$ antibodies does not enhance the maximal activation of NK activity seen following either stimulus alone (data not shown). Further studies will be necessary to explore the different roles and potential mechanisms of interaction between IL-2 and T11 activation in the regulation of NK activity.

Another important issue addressed by the present studies is the identification of those cells capable of mediating cytotoxicity following in vitro activation. Other investigators have previously demonstrated that some cells in normal peripheral blood can be induced to spontaneously kill a variety of target cells following incubation with various lymphokines and clinical studies utiliz-

ing in vitro activated cytotoxic cells in patients with metastatic cancer have recently been reported [15, 16]. These cells have been termed lymphokine activated killer (LAK) cells [4] under the assumption that they are different from both T cells and NK cells. The results presented in this report consistently demonstrate that spontaneous cytotoxicity against a variety of target cells is exclusively contained within the $NKH1^+$ population of PBMC. In vitro activation by either IL-2 or anti-$T11_{2/3}$ results in marked enhancement of cytotoxicity against NK-sensitive targets as well as simultaneous induction of cytotoxicity against previously NK-resistant targets. However, with both stimulated and unstimulated effector cells, enhanced cytotoxicity remains confined to the $NKH1^+$ population which only represents a small fraction (approximately 12%) of PBMC. These studies therefore strongly suggest that LAK is a direct result of the activation and proliferation of $NKH1^+$ natural killer cells and argues against a separate lineage derivation of these cells [20]. In contrast, our studies support the view that the LAK phenomenon primarily reflects the functional effects of various lymphokines on the regulation of NK activity in vivo.

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