

THE ACTIVITY OF NATURAL CYTOTOXIC CELLS IS
AUGMENTED BY INTERLEUKIN 2 AND INTERLEUKIN 3*

BY EDMUND C. LATTIME, GENE A. PECORARO, AND OSIAS STUTMAN

From the Cellular Immunology Section, Memorial Sloan-Kettering Cancer Center, New York 10021

Natural cell-mediated cytotoxicity (NCCMC) has been defined as the ability of lymphoid cells derived from normal donors to lyse a number of tumor target cells in vitro (1, 2). In mice, at least two distinct NCCMC effector systems have been identified: natural killer (NK) (1, 2) and natural cytotoxic (NC) cells (3). NK cells were first described by their cytotoxicity against lymphoid tumor targets (1, 2) and subsequently shown to lyse solid tumors as well (4). NC cells were described by our laboratory and shown primarily to lyse nonlymphoid solid tumor cell lines (3-5). NK and NC cells are active in the absence of known priming, present in athymic nude mice, of a still undefined lineage, and do not have properties of conventional T cells, B cells, or macrophages (1-7). However, NK and NC cells differ in strain distribution of activity and expression of cell surface antigens (1-8). NK cell activity is augmented by interferon (IFN), IFN-inducing agents (9), and the lymphokine interleukin 2 (IL-2) (10, 11). NC cell activity is not augmented by IFN or IFN-inducing agents (4), and when augmented lysis is detected against a particular NC-susceptible target, it is due to a superimposed NK-augmented component (4).

In the present study we show that in vitro exposure to IL-2 augments both NK and NC activity, while another lymphokine or monokine, interleukin 3 (IL-3) (12), augments only NC activity. Conversely, and included for the sake of comparison, the IFN-inducer poly-IC augments NK but not NC cell activity. We also show here that the IL-2 augmented NK and NC cytotoxic activities are the function of precursor and effector cells with different expression of the Qa 5 marker, which is present on spontaneous and augmented NK cells (13), but not on NC cells (8). This supports our contention that NCCMC is mediated by at least two distinct subpopulations of related but distinct effector cells.

Materials and Methods

Animals. All experiments were done with spleen cells from 6-8-wk-old C57BL/6J mice of both sexes, obtained from The Jackson Laboratory, Bar Harbor, ME.

Target Cells and Cell Preparation Procedures. The YAC-1 lymphoma targets were obtained in 1978 from the late Dr. G. Cudkovicz and kept in culture as described (8). The WEHI-164, a chemically induced fibrosarcoma of BALB/c origin that is an exclusive NC target (14, see also ref. 4) was obtained from Dr. R. C. Burton when at the Massachusetts General Hospital, Boston, MA, and kept in culture as described (4). Spleen cell suspensions for the assays were prepared as before (3).

Assay Systems. NK activity was tested against YAC-1 and NC activity was tested against WEHI 164 targets, using a standard 18-h ⁵¹Cr release assay, as described (4).

* Supported by grants CA-08748, CA-15988, CA-17818, and CA-25932 from the National Institutes of Health, and grant IM-188 from the American Cancer Society.

TABLE I
Augmentation and Mediation of NC and NK Cell Activity

| Group | Target | Effectors* | Percent cytotoxicity‡ | | | |
|-------|----------|------------|-----------------------|------|----------------|------|
| | | | Control‡ | | Anti-Qa-5 + C§ | |
| | | | 100:1 | 50:1 | 100:1 | 50:1 |
| 1 | YAC-1 | Fresh NK | 37 | 26 | 0 | 0 |
| | | 24 h | 0 | 0 | 0 | 0 |
| | | Poly-IC | 34 | 32 | 1 | 4 |
| 2 | YAC-1 | Fresh NK | 23 | 17 | 8 | 6 |
| | | 24 h | 9 | 7 | 4 | 4 |
| | | IL-2 | 42 | 38 | 9 | 4 |
| 3 | WEHI-164 | IL-3 | 12 | 7 | 4 | 5 |
| | | Fresh NC | 24 | 18 | ND¶ | ND |
| | | 24 h | 23 | 17 | ND | ND |
| 4 | WEHI-164 | Poly-IC | 32 | 16 | ND | ND |
| | | Fresh NC | 52 | 35 | 47 | 31 |
| | | 24 h | 37 | 29 | 47 | 33 |
| | | IL-2 | 74 | 53 | 70 | 48 |
| | | IL-3 | 62 | 56 | 65 | 55 |

* C57BL/6J spleen cells either fresh or cultured as indicated alone for 24 h or with poly-IC at 25 µg/ml (poly-IC), IL-2 (at 20 U/ml), or IL-3 (at 20 ED50/ml) for 24 h (see Materials and Methods for further details) were tested for NK and NC activity in 18 h ⁵¹Cr-release assays against YAC-1 (an NK-susceptible target) and WEHI-164 (an NC-susceptible target, resistant to NK lysis; see ref. 4 and 14). For further details on assays, see Materials and Methods.

‡ Percent cytotoxicity calculated as in Material and Methods.

§ These cells were tested in the NK-NC assay against YAC-1 or WEHI-164 targets after in vitro treatment with anti-Qa5 monoclonal and C (see Materials and Methods for details).

|| Significant differences, $P < 0.05$ from the 24 h control.

¶ Not done.

Preincubations of Effector Cells. Spleen cells were preincubated for 24 h in the same medium as used for the assays (4), either alone (indicated in Table I as "24 h") or in the presence of 25 µg/ml of poly-IC (4) or 20 U/ml of IL-2 or IL-3 (20 ED50/ml) at cell concentrations of 5×10^6 /ml. The IL-2 was semi-purified from Con-A-stimulated rat spleen cell cultures, with the mitogen removed by ammonium sulfate precipitation and Sephadex G-100 chromatography (15). The units defined as in refs. 15 and 16. The IL-3 was provided by Dr. J. N. Ihle, Frederick Cancer Center, Frederick, MD, and purified to homogeneity from WEHI-3 supernatants (12). IL-3 was received at a concentration of 10^3 ED50/ml in saline and further diluted in the medium described above. For definition of IL-3 units see ref. 12. The IL-2 preparation used in these experiments had no IL-3 activity as measured by its ability to support the proliferation of an IL-3-dependent cell line 32D cl 5, obtained from Dr. J. S. Greenberger, Sidney Farber Cancer Center, Boston, MA, using a procedure developed in our laboratory (R. A. Miller and O. Stutman, manuscript in preparation). Similarly, the IL-3 preparation had no detectable IL-2 activity when measured in a similar fashion, using an IL-2-dependent T cell line (15, 16).

Treatment of Cells with Anti-Qa5. The monoclonal antibody anti-Qa5 was obtained from Dr. U. Hammerling of Memorial Sloan-Kettering Cancer Center and used for mass lysis of cells (4). The effector cells were either treated with anti-Qa-5 and C as fresh cells or after the 24 h preincubations, as described in Tables I and II.

Results

Table I shows the effects of IL-2, IL-3, and poly-IC on spleen NK and NC activity. Groups 1 and 2 show that NK cell activity, measured as lysis of the lymphoma YAC-1 in an 18-h ⁵¹Cr release assay, was significantly augmented by preincubation of the cells with poly-IC or IL-2 for 24 h, when compared with cells incubated alone

TABLE II
Pretreatment of Spleen Cells with Anti-Qa5 and C Removes the Poly-IC and IL-2 Augmentable NK Precursor While Not Affecting the IL-2 and IL-3-responsive NC Precursor Population

| Group | Target | Effector* | Percent cytotoxicity‡ | | |
|-------|----------|--------------------|-----------------------|------|------|
| | | | 100:1 | 50:1 | 25:1 |
| 1 | YAC-1 | Fresh NK | 23 | 17 | ND§ |
| | | 24 h | 9 | 7 | ND |
| | | IL-2 | 42 | 38 | ND |
| | | Pre-Qa-5 + C | 6 | 4 | ND |
| | | Pre-Qa-5 + IL-2 | 6 | 4 | ND |
| 2 | YAC-1 | Fresh NK | ND | 10 | 8 |
| | | 24h | ND | 0 | 0 |
| | | Poly-IC | ND | 24 | 19 |
| | | Pre-Qa-5 + C | ND | 0 | 0 |
| | | Pre-Qa-5 + poly-IC | ND | 1 | 0 |
| 3 | WEHI-164 | Fresh NC | 52 | 35 | ND |
| | | 24 h | 37 | 30 | ND |
| | | IL-2 | 74 | 53 | ND |
| | | IL-3 | 62 | 56 | ND |
| | | Pre-Qa-5 + C | 43 | 30 | ND |
| | | Pre-Qa-5 + IL-2 | 61 | 47 | ND |
| | | Pre-Qa-5 + IL-3 | 56 | 44 | ND |

* C57Bl/6J spleen cells fresh, or cultured as indicated were used as effectors for the assays (fresh, cultured alone for 24 h, (denoted 24 h), cultured with poly-IC (poly-IC), IL-2 (IL-2) or IL-3 (IL-3) for 24 h, and spleen cells pretreated with anti-Qa-5 + C cultured alone (Pre-Qa-5 + C) or with the agents described above).

‡ All studies were done using 18-h ⁵¹Cr assays. For description of the assay and effector cells see Materials and Methods.

§ Not done.

|| *P* < 0.05 significantly different from control (24 h or pre-Qa-5 + C).

for 24 h (which showed the characteristic loss of NK activity) (1, 2, 5), and comparable to the values observed with fresh NK cells. Little if any effect of IL-3 on NK cell activity was noted (group 2, Table I). Table I also shows that the augmented lysis of the YAC target by the NK cells treated with poly-IC or IL-2 is the function of a Qa-5⁺ effector cell, as is the case for fresh NK cells (8, 13).

When similarly pretreated spleen cells were assayed for their capacity to lyse the NC-sensitive, NK-resistant fibrosarcoma WEHI-164 in an 18-h ⁵¹Cr release assay (groups 3 and 4 in Table I), a different pattern of activities was noted. Poly-IC failed to induce any increase in NC cell activity (4), whereas both IL-2 and IL-3 treatment lead to a significant augmentation of lysis of the NC-susceptible target (group 4 in Table I). The augmented activity after IL-2 or IL-3 treatment was consistently above the values observed with either spleen cells incubated alone for 24 h or fresh spleen NC cells. Groups 3 and 4 in Table I also show that the activity of fresh NC cells is comparable to that of cells incubated alone for 24 h, a stability in culture (3, 5, 18) that contrasts with the labile NK cells (1, 2, 5, 18). As also shown in groups 3 and 4 in Table I and in contrast with the NK cell findings, the effector cells responsible for the augmented lysis of the WEHI-164 target induced by both IL-2 and IL-3 were Qa-5⁻, as was the case for fresh spleen NC cells (8). This type of result with NK and NC cells was observed in each of five experiments. In summary, NK activity, both

spontaneous and augmented (by poly-IC and IL-2) was mediated by a $Qa-5^+$ effector, whereas the spontaneous or augmented (by IL-2 or IL-3) NC activity was the function of a $Qa-5^-$ effector cell.

Table II shows that the difference in $Qa-5$ surface phenotype between the NK and NC effector cells is also observed at the level of the augmentable precursor cells. Pretreatment of the spleen cells with anti- $Qa-5$ and C before the culture with poly-IC or IL-2 completely abrogated the augmentation of NK cell activity (groups 1 and 2 in Table II); thus, the augmentable NK precursor is $Qa-5^+$, as observed in studies using IFN (17). Conversely, group 3 in Table II shows that pretreatment of the spleen cells with anti- $Qa-5 + C$ had no effect on the augmentation of NC cell activity by IL-2 and IL-3; thus, the augmentable NC precursor is $Qa-5^-$, as is the case for the spontaneous and augmented NC effector (8) (Table I).

Discussion

The results presented here add two more properties to support our views on the heterogeneity of effector cells mediating NCMC (3). They show that although a factor such as IL-2 can augment both NK and NC activities, it does so by acting on distinct cell populations ($Qa-5^+$ vs. $Qa-5^-$). Our results also show that the cytotoxic activity of NK and NC cells are affected by different factors, with poly-IC (and IFN) (4, 9, 17) augmenting NK but not NC activity, and IL-3 (12) augmenting NC but not NK activity.

Although we (3-8, 18) and others (14, 17, 19) have described a variety of properties of the NC system, we have been unable to ascribe the NC cell to a particular lineage. A similar problem remains with the NK cell (1, 2, 20). Although we have been unable to identify any NC-associated cell surface markers, we do know that NC cells lack T, B, NK, and macrophage-associated antigens (5, 8, 18). It is tempting to speculate from the present findings that since both IL-2 and IL-3 are soluble factors associated with some stages of T cell differentiation or function (12, 21), and both factors augment NC cell activity, NC cells may be related in some way to the T lineage. Without further evidence, however, this remains only a speculative proposition at present.

In general, IL-2 induces responses that have been associated with $Thy-1^+$ populations, both in T (21) and NK studies (17). Our demonstration that IL-2 also augments the activity of NC cells, which are $Thy-1^-$ (5, 18), raises several possible interpretations: (a) IL-2 may possess a broader range of activities than thus far described, as seems the case for other hemopoietic growth factors (22); (b) The activity that we have ascribed to augmented NC cells may be due to a $Thy-1^+ Qa-5^-$ population of the anomalous killer (AK) type (23). The fact that the IL-2 augmentable and effector cell in our system are both $Qa-5^-$ would eliminate the possibility of it being a cell of the NK_T type described by Minato et al. (17). (c) the IL-2 preparation used has other factors besides IL-2. Our previous studies, as well as those described in this report, would suggest the first interpretation, although the third interpretation cannot be completely excluded. We have previously reported that removal of $Thy-1^+$ cells from spleen or marrow had no effect on NC cell activity (5, 18), and pretreatment of spleen cells with monoclonal anti- $Thy-1$ or anti- $Lyt-2$ and C does not affect the augmentation of NC activity by IL-2 (our unpublished results). Furthermore, as shown in Tables I and II, IL-2 treated $Qa-5^-$ cells show augmented lysis only of the NC target, while failing to lyse the YAC-1 line. It is doubtful that an AK-like cell would show such NC target

preference (23). The possibility that factors other than IL-2 might be present in the IL-2 used cannot be ruled out. However, since as described above, the IL-2 preparation used did not contain any IL-3 activity, we may conclude that the augmented NC cell activity is probably a function of the IL-2 in our preparation.

In addition to the apparent capacity to augment NC cell activity, IL-3 has been shown to have at least two effects: it can induce the expression of the T cell-associated enzyme 20-alpha-hydroxysteroid dehydrogenase, probably related to some early stages of thymocyte and T cell development (12) and can promote the growth of IL-3-dependent cell lines with either a T-like or a "mast cell"-like phenotype (12). A comparison between NC cells and some of the IL-3-responsive populations or IL-3-dependent cell lines shows some similarities, i.e., tissue distribution and lack of some surface markers (3, 5, 8, 12), but they are far from conclusive, although they suggest that NC cells and other IL-3-responsive populations may be related, especially since some of the IL-3 dependent cell lines (12) appear to have NC-like cytotoxicity (24).

Three conclusions regarding heterogeneity of NCMC effector cells can be derived from the present studies. First, the demonstration that NC and NK cells differ in their regulation strengthens our conclusions that the two activities are functions of distinct, although perhaps related, cellular populations. Second, the demonstration that a second NCMC effector system, distinct from NK, and lacking T cell markers, is regulated by IL-2 tentatively suggests that the lymphokine may have a broader range of activities than previously described (an alternative view being that both NC and NK systems are affected by IL-2 because they are somewhat related to the T lineage). Third, the augmentation of NC cell activity by IL-3 suggests that NC cells may belong to the group of IL-3 dependent cells (12), although it is still not clear from this fact whether they belong to any of the particular cell lineages that are affected by IL-3.

Summary

Murine natural killer (NK) and natural cytotoxic (NC) cells showed different patterns of augmentation of lytic activity after preincubation for 24 h with either poly-IC, interleukin 2 (IL-2), or interleukin 3 (IL-3): (a) Poly-IC augmented only NK cells, with no effect on NC activity, as we have previously observed (4); (b) IL-2 augmented both NK and NC activity; and (c) IL-3 augmented only NC lysis, without affecting NK activity. In addition, both precursor and the augmented effector cells showed differences in expression of the Qa-5 surface marker: NK precursors and effectors are Qa-5⁺, whereas NC precursors and effector cells are Qa-5⁻.

Received for publication 9 November 1982 and in revised form 6 December 1982.

References

1. Herberman, R. B., and H. T. Holden. 1978. Natural cell-mediated immunity. *Adv. Cancer Res.* **27**:305.
2. Roder, J. C., K. Karre, and R. Kiessling. 1981. Natural killer cells. *Prog. Allergy.* **28**:66.
3. Stutman, O., C. J. Paige, and E. Feo Figarella. 1978. Natural cytotoxic cells against solid tumors in mice. I. Strain and age distribution and target cell susceptibility. *J. Immunol.* **121**:1819.
4. Lattime, E. C., G. Pecoraro, M. Cuttito, and O. Stutman. 1982. Solid tumors are killed by NK and NC cell populations: interferon inducing agents do not augment NC cell activity under NK activating conditions. *In* NK Cells and Other Natural Effector Cells. R. B. Herberman, editor. Academic Press, Inc., New York. 179.

5. Paige, C. J., E. Feo Figarella, M. J. Cuttito, A. Cahan, and O. Stutman. 1978. Natural cytotoxic cells against solid tumors in mice. II. Some characteristics of the effector cells. *J. Immunol.* **121**:1827.
6. Stutman, O., P. Dien, R. A. Wisun, and E. C. Lattime. 1980. Natural cytotoxic cells against solid tumors in mice: blocking of cytotoxicity by D-mannose. *Proc. Natl. Acad. Sci. USA.* **77**:2895.
7. Stutman, O., and M. J. Cuttito. 1981. Normal levels of natural cytotoxic cells against solid tumors in NK-deficient beige mice. *Nature (Lond.)*. **290**:254.
8. Lattime, E. C., G. A. Pecoraro, and O. Stutman. 1981. Natural cytotoxic cells against solid tumors in mice. III. A comparison of effector cell antigenic phenotype and target cell recognition structures with those of NK cells. *J. Immunol.* **126**:2011.
9. Djeu, J. Y., J. A. Heinbaugh, H. T. Holden, and R. B. Herberman. 1979. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. *J. Immunol.* **122**:175.
10. Henney, C. S., K. Kuribayashi, D. E. Kern, and S. Gillis. 1981. IL-2 augments natural killer cell activity. *Nature (Lond.)*. **291**:335.
11. Kuribayashi, K., S. Gillis, D. E. Kern, and C. S. Henney. 1981. Murine NK cell cultures: effects of IL-2 and interferon on cell growth and cytotoxic reactivity. *J. Immunol.* **126**:2321.
12. Ihle, J. N., L. Rebar, J. Keller, J. C. Lee, and A. Hapel. 1982. Interleukin-3: possible roles in the regulation of lymphocyte differentiation and growth. *Immunol. Rev.* **63**:1.
13. Chun, M., V. Pasanen, U. Hammerling, G. Hammerling, and M. K. Hoffman. 1979. Tumor necrosis factor induces a serologically distinct population of NK cells. *J. Exp. Med.* **150**:426.
14. Burton, R. C. 1980. Alloantisera selectively reactive with NK cells: characterization and use in defining NK cell classes. In *Natural Cell-mediated Immunity against Tumors*. R. B. Herberman, editor. Academic Press, Inc., New York. 19.
15. Miller, R. A., and O. Stutman. 1982. Enumeration of IL-2 secreting helper T cells by limiting dilution analysis, and demonstration of unexpected high levels of IL-2 production per responding cell. *J. Immunol.* **128**:2258.
16. Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* **120**:2027.
17. Minato, N., L. Reid, and B. R. Bloom. 1981. On the heterogeneity of murine natural killer cells. *J. Exp. Med.* **154**:750.
18. Stutman, O., E. Feo Figarella, C. J. Paige, and E. C. Lattime. 1980. Natural cytotoxic (NC) cells against solid tumors in mice: general characteristics and comparison to natural killer (NK) cells. In *Natural Cell-mediated Immunity against Tumors*, R. B. Herberman, editor. Academic Press, Inc., New York. 187.
19. Lust, J. A., V. Kumar, R. C., Burton, S. B. Bartlett, and M. Bennett. 1981. Heterogeneity of natural killer cells in the mouse. *J. Exp. Med.* **154**:306.
20. Saksela, E., T. Timonen, A. Ranki, and P. Hayry. 1979. Morphological and functional characterization of isolated effector cells responsible for human natural killer activity to fetal fibroblasts and to cultured cell line targets. *Immunol. Rev.* **44**:71.
21. Smith, K. A., and F. W. Ruscetti. 1981. T cell growth factor and the culture of cloned functional T cells. *Adv. Immunol.* **31**:137.
22. Iscove, N. N., C. A. Roitsch, N. Williams, and L. J. Guilbert. 1982. Molecules stimulating early red cell, granulocyte, macrophage, and megakaryocyte precursors in culture-similarity in size, hydrophobicity and charge. *J. Cell. Physiol.* **1**(Suppl.):65.
23. Karre, K., and J. K. Seeley. 1979. Cytotoxic Thy 1.2 positive blasts with NK-like target selectivity in murine mixed lymphocyte cultures. *J. Immunol.* **123**:1511.
24. Djeu, J. Y., E. Lanza, A. J. Hapel, and J. Ihle. 1982. Natural cytotoxic activity of mouse spleen cell cultures maintained with interleukin-3. In *NK Cells and Other Natural Effector Cells*. R. B. Herberman, editor. Academic Press, Inc., New York. 917.