dients can be estimated, regardless of the paleogeography employed, by taking note of the presence or absence of widespread, sea level, continental glaciation (such as that of the later Ordovician and earliest Silurian, and the Late Carboniferous and Lower Permian), and the size of the areas occupied by cool- to cold-climate type sediments and fossils (Atlantic, Malvinokaffric, and Gondwana) as contrasted with warm to hot types (Pacific, Paleotethyan, North Siluri-an realm, and Old World realm) during the Paleozoic (*3*).
14. Isotopic paleothermometry is available for Cenozoic and younger Mesozoic deposits, but suitable material is sparse prior to the Jurassic. The cost in time and money has prevented paleothermometry from becoming widely used for routine purposes in the pre-Triassic.
15. A. A. Meyerhoff and C. Teichert [*J. Geol.* **79**, 285 (1971)] cite Gondwana evidence of preserved montane glaciation in the form of glacial valleys.
16. There are major changes in level of biogeographic provincialism within the following Paleozoic periods (*3*): Ordovician (Early Ordovician, highly provincial; Middle and Late Ordovician, moderately provincial); Devonian (Early and early Middle Devonian, highly provincial; remainder of Middle Devonian, moderately provincial; and Late Devonian, highly cosmopolitan); Carboniferous (Early Carboniferous, highly cosmopolitan; Late Carboniferous, moderately provincial).
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RESEARCH ARTICLE

Homeostasis of the Antibody Response: Immunoregulation by NK Cells

Lynne V. Abruzzo and Donald A. Rowley

The primary antibody response to most bacterial, viral, and other antigens is characterized by an exponential increase in the number of B lymphocytes secreting specific immunoglobulin M (IgM) antibody (direct plaque-forming cells or PFC). The increase in PFC begins about 1 day and terminates 4 to 6 days after immunization (*1*). The mechanism of termination has been variously attributed to: (i) exhaustion of antigen, (ii) terminal differentiation of essential cells, (iii) antibody feedback suppression, (iv) anti-idiotypic feedback suppression, or (v) suppressor T cells. However, the first two mechanisms are not supported by experimental evidence, and antibody, anti-idiotypic antibody, and suppressor T cells are only potent and specific suppressors when given to animals or added to cultures just before or at the time of immunization, not when given two or more days after immunization (*2*). Thus the mechanism of termination is an enigma.

Abstract. *When injected into mice, the synthetic double-stranded polynucleotide poly(inosinic) · poly(cytidylic) acid induces high natural killer (NK) cell activity within 4 to 12 hours. Induction of NK activity in mice immunized 2 or 3 days previously, or the addition of NK cells to cultures immunized in vitro 2 or 3 days previously, promotes early termination of the ongoing primary immunoglobulin M antibody response. A target for NK cells is a population of accessory cells that has interacted with antigen and is necessary for sustaining the antibody response. The inference is strong that NK cells induced normally by immunization also terminate the usual antibody response in vivo by elimination of antigen-exposed accessory cells.*

We decided to explore an unconventional mechanism of regulation when we observed the kinetics of the response of purified B cells to a T-cell independent antigen on adoptive transfer to lethally irradiated (600 R) mice. Both the rate and duration of increase and decrease in PFC paralleled that observed in intact mice. In a large series of experiments we could not account for termination of the response of the adoptively transferred cells by any of the usual mechanisms and were led, therefore, to consider the possibility that regulation might be mediated by an x-ray–resistant, non-B–non-T-cell population present in x-irradiated recipients. Cells that are relatively resistant to x-irradiation include the heterogeneous population of cells that have the gross morphology of lymphocytes but are nonspecifically cytotoxic for diverse, virally infected, or tumor cells. Such cytotoxic cells, present in normal and nude or athymic mice that have had no known exposure to the target antigen, are referred to as natural killer (NK) cells (*3*). Natural killer cells are themselves a heterogeneous population, but for our purposes NK cells are: x-ray resistant, nonadherent to plastic culture dishes or to carbonyl iron particles, cytotoxic for YAC-1 tumor target cells, inducible by poly(inosinic) · poly(cytidylic) acid [poly(I) · poly(C)], and are cells whose cytotoxic activity is eliminated by an-

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tibodies to NK cells and complement (*4–6*).

First, we determined that poly(I) · poly(C) injected into mice immunized 2 or 3 days earlier with phosphorylcholine of R36a pneumococcal vaccine or sheep erythrocytes (SRBC) (*7*) induced increased NK activity and produced early termination of PFC responses (Table 1).

Presumably, NK cells injected into immunized mice might reproduce the effect of giving poly(I) · poly(C); however, the homing of injected NK cells to the spleen is very low (*8*), so we elected to study the phenomenon in vitro using cultures of (i) normal spleen cells immunized in vitro 2 or 3 days previously and (ii) spleen cells obtained from mice immunized in vivo 2 or 3 days previously and cultured with the original immunizing antigen. Responses were measured 3 to 6 days after original immunization of cultures or animals; peak responses and the greatest differences between treatment groups usually occurred 4 or 5 days after original immunization with phosphorylcholine and 5 or 6 days after original immunization with SRBC; otherwise, all of the essential findings were similar regardless of the antigen or culture system used.

Poly(I) · poly(C) added in quantities of 0.01, 0.1, 1.0, 10, or 100 μg per culture of 200 μl of immunized cells had no effect on PFC whether the cultures were assayed 1, 24, or 48 hours after the addition. In contrast, critical numbers of cells enriched for NK activity added to cultures caused 50 percent or greater loss of PFC within 24 to 48 hours. For example, in one experiment, 10^5 cells enriched for NK activity (*5*) added to cultures of 10^6 cells immunized in vitro 2 days previously with phosphorylcholine reduced the mean number of PFC per culture from 108 to 14 measured at 4 days; in another experiment, the same number of cells added to cultures immunized with SRBC 2 days previously reduced the mean number of PFC from 164 to 76 measured at 5 days. While this effect of added cells enriched for NK was regularly reproducible, the critical number required varied from experiment to experiment from 1×10^5 to 5×10^5 cells per culture. Furthermore, in many experiments a smaller number of cells than the critical number resulted in significantly higher numbers of PFC per culture. Apparently, the preparations of NK cells included both suppressors (presumably NK cells) and accessory (A) cells, even though we attempted to remove A cells by adhering them to the bottoms of plastic culture dishes or to the carbonyl iron particles. The likelihood of this explanation being correct was supported by the observation that treating preparations of NK cells with antibody to NK and complement eliminated suppression and improved the effectiveness of added cells to increase the numbers of PFC in cultures (Table 2).

Presumably, the target for NK cells could be either responding lymphocytes or A cells, or both (*9*). Both populations are required for induction of primary responses (*10–12*) and we find that A cells are also required for sustaining an ongoing PFC response of cells obtained from mice immunized 2 or 3 days previously (*13*). For example, A cells were removed from suspensions of spleen cells obtained from mice 2, 3, or 4 days after immunization; the cells were cultured with antigen and the number of PFC were assayed immediately and 24 and 48 hours later. The number of PFC dropped precipitously to 10 to 50 percent of the original number after 24 hours in culture and usually to less than 10 percent by 48 hours. The addition of A cells and antigen to suspensions depleted of A cells greatly reduced or prevented the loss so that the numbers of PFC usually increased after 2 or 3 days in culture. Thus, the number of PFC in cultures was a complex function of survival and proliferation of PFC obtained from immunized mice. We found that A cells obtained from either unimmunized mice or mice immunized 2 or 3 days previously were effective in preventing loss of PFC. Addition of antigen was required for preventing the loss of PFC regardless of the source of A cells, but antigen alone was not effective.

The requirement for A cells for survival and proliferation of PFC is shown in Table 3. In experiment 1 of this table, PFC and A cells are compared with the same populations obtained from mice injected with poly(I) · poly(C). While A cells obtained from immunized mice prevented PFC loss, A cells from immunized mice injected with poly(I) · poly(C) did not prevent loss of PFC. In experiment 2 (Table 3), the deficit in A cells obtained from poly(I) · poly(C) treated immunized mice was restored by adding to cultures a suspension of A cells from non–poly(I) · poly(C)–treated mice. In other experiments of similar design we determined that the deficit in A cells from poly(I) · poly(C)–treated mice was not due to contaminating NK cells because treatment of A cells with antibody to NK cells (antibody to asialo G_{M1}) and complement did not restore activity (nor did such antibody treatment decrease the activity of normal A cells).

In experiment 1 of Table 3, the disappearance of PFC in cells obtained from mice injected with poly(I) · poly(C) was equivalent to that observed for PFC in cells obtained from mice not injected with poly(I) · poly(C); that is, PFC disappeared rapidly in the absence of A cells and did not disappear in the presence of effective A cells and antigens. Although this observation did not rule out PFC (or helper T cells) as a target for NK cells, the findings suggested that the

Table 1. Poly(inosinic) · poly(cytidylic) acid–induced NK activity and promoted early termination of the antibody (PFC) response. Mice (10 to 12 weeks old) were injected intravenously with 0.2 ml of R36a vaccine (approximately 10^8 killed bacteria) and intraperitoneally with 100 μg of poly(I) · poly(C) in 0.2 ml of saline. Cytotoxicity was measured against ^{51}Cr-labeled YAC-1 target cells (*5*) over a range of effector cell (whole spleen cells) to target cell (E:T) ratios of 4:1 to 250:1. In other experiments, as well as this one, the cells from 4-day immunized, non–poly(I) · poly(C)–treated mice invariably released < 50 percent ^{51}Cr at all E:T ratios, whereas cells from poly(I) · poly(C)–treated mice invariably released > 50 percent ^{51}Cr at E:T ratios of 32:1 to 125:1. Splenic PFC were assayed in Cunningham chambers against phosphorylcholine coupled to SRBC. Each number of PFC recorded is the mean for the group calculated from counts of replicate samples of spleen cells obtained from each mouse; in each experiment differences between groups were significant, $P = < 0.05$. PFC levels assayed on days 5 and 6 showed a progressive, parallel decline for untreated and poly(I) · poly(C)–treated mice.

Day 0: immunization with R36a	Day 3: NK activity induced by poly(I) · poly(C)	Day 4: response					
		Cytotoxicity of spleen cells determined for different E:T ratios in experiment 1 (N = 3)			PFC per spleen		
		250:1	125:1	64:1	Experiment 1 (N = 3)	Experiment 2 (N = 2)	Experiment 3 (N = 4)
+	0	28	17	13	217,000	123,000	69,000
+	+	70	57	38	88,000	28,000	14,000
0	0	34	29	18			
0	+	79	65	52			

observed effects of poly(I) · poly(C) could be accounted for by changes produced in the population of A cells.

It was interesting that A cells prepared from unimmunized mice injected with poly(I) · poly(C) 12 to 24 hours before they were killed were invariably as effective or more effective in preventing PFC loss than A cells obtained from normal mice or from mice immunized 2 days previously but not injected with poly(I) · poly(C). This finding suggested that only A cells that have interacted with antigen in the process of immunization were a target for NK cells. This possibility was tested by adding NK cells to A cells that had been briefly exposed to antigen. In some experiments A cells were prepared from spleens of unimmunized mice; the cells adherent to culture wells were incubated with antigen for 1.5 hours before they were washed and NK cells were added. In other experiments, A cells were prepared from spleens of mice immunized 2 or 3 days previously; in these experiments, NK cells were added directly to adherent cells. In both kinds of experiments, NK cells were incubated with A cells for 4 hours and were then removed by washing the cultures. Suspensions of PFC (with A cells removed) obtained from spleens of mice immunized 3 days previously were dispensed along with the original immunizing antigen to cultures that were assayed 1, 2, and 3 days later. As shown in Table 4, and in a subsequent series of 11 consecutive experiments, NK cells invariably reduced by 30 to 80 percent the capacity of antigen-exposed A cells to sustain PFC whether prior exposure of A cells to antigen occurred in vitro or in vivo.

In contrast, the effect of adding NK cells to A cells that had not been exposed to antigen, that is, A cells prepared from spleens of normal mice, was variable (data not shown); in some experiments A cells that had been incubated with NK cells were normally effective while in other experiments NK cells reduced the effectiveness of non-antigen–exposed A cells, possibly because the fetal calf serum in the medium contained an antigen or because NK cells were not effectively removed by washing before the final addition of PFC and antigen. In repeated experiments, the effect of incubating A cells with NK cells was eliminated by either reducing by ten times the number of cells enriched for NK or by treating the suspensions with antibody to NK and complement. In fact, NK cell preparations diluted or treated with antibody to NK cells and complement usually caused significantly higher PFC responses than antigen-exposed A cells alone, again indicating that all A cells were probably not removed from our preparations of NK cells. Thus, the findings strongly support the likelihood that A cells that have interacted with antigen are a target for NK cells.

Presumably A cells required for both inducing and sustaining PFC responses include A cells that produce interleukin-1, present antigen, and are directly or indirectly involved in generating various other factors responsible for proliferation and antibody secretion by PFC. At present we do not know whether a single subpopulation or multiple subpopulations of A cells are deficient in immunized mice treated with poly(I) · poly(C) or are the target for NK cells in vitro.

The NK system has presumably evolved as a line of defense against an

Table 2. Suppression of the ongoing antibody response in vitro by cells enriched for NK activity. Whole spleen cells, 10^6 cells per culture, were immunized with R36a vaccine. Cells enriched for NK activity were prepared from C3H mice injected with poly(I) · poly(C) 24 hours before and irradiated with 600 R 12 hours before they were killed. The cell preparation was incubated with carbonyl iron for 1 hour (*12*) to remove adherent cells. One-half of the preparation, at a cell concentration of 10^7 cells per milliliter, was incubated with antiserum to NK cells and rabbit complement, final dilutions of 1:100 and 1:10, respectively, for 1 hour at 37°C; the treated cells were washed twice; the untreated cells were similarly incubated and washed. Treated and untreated cells were assayed for cytotoxicity against ^{51}Cr-labeled YAC-1 cells and were added to cultures as indicated. The untreated cells caused 80 percent lysis at an E:T of 50:1 and 50 percent lysis at an E:T ratio of 6:1, whereas the treated cells caused < 40 percent lysis at an E:T ratio of 100:1 and < 10 percent lysis at an E:T of 6:1. Each number recorded is the average count per culture calculated from counts of PFC in duplicate Cunningham chambers prepared from a pool of three replicate cultures.

Additions to cultures on day 3	Number of PFC per culture on day 5
Medium	149
NK enriched cells (4×10^5)	36
NK enriched cells (4×10^4)	81
NK enriched cells (4×10^5) treated with antibody to NK and complement	384
NK enriched cells (4×10^4) treated with antibody to NK and complement	189

Table 3. A cells from mice immunized 3 days previously prevented the loss of PFC, but A cells from similarly immunized mice given poly(I) · poly(C) did not. The A cells and PFC were prepared from mice immunized with 0.2 ml of 5 percent SRBC 3 days previously. Donors of PFC were either untreated or injected 24 hours before they were killed with 100 μg of poly(I) · poly(C); A cells were removed from suspensions of PFC by incubation with carbonyl iron. Cell concentrations were adjusted to 10^5 cells per 100 μl and PFC were assayed; 10^5 cells contained approximately 50 PFC in experiment 1 and 200 PFC in experiment 2. Donors of A cells were either untreated or injected 24 hours before they were killed with 100 μg of poly(I) · poly(C); all donors of A cells received 600 R 12 hours before they were killed. In both experiments, suspensions of A cells were adjusted to 10^5 cells per 100 μl per culture well; cultures were incubated for 1.5 hours and then washed twice to remove nonadherent cells. To each culture we added 100 μl of suspension containing PFC and 10 μl containing approximately 5×10^7 SRBC. In experiment 2, an additional 50 μl of a suspension containing 10^5 A cells prepared from untreated x-irradiated normal mice were added to each culture in the last group. The results recorded are for cultures assayed 2 days later, that is, 5 days after initial immunization in vivo. Each number recorded is the mean count per culture calculated from counts of PFC in duplicate Cunningham chambers prepared from a pool of three replicate cultures.

Additions to cultures	Number of PFC per culture	
	PFC from untreated donors	PFC from donors treated with poly(I) · poly(C)
Experiment 1		
No A cells	7	7
A cells from immunized mice given poly(I) · poly(C)	16	11
A cells from immunized mice	65	65
Experiment 2		
No A cells	13	
A cells from immunized mice given poly(I) · poly(C)	42	
A cells from immunized mice	232	
A cells from immunized mice given poly(I) · poly(C) plus A cells from normal mice	178	

Table 4. NK cells added briefly to A cells that had been exposed to antigen reduced the survival and proliferation of PFC added subsequently. See text for general experimental outline. In experiment 1, A cells were prepared from spleens of normal mice irradiated with 600 R 12 hours before they were killed. Cells were dispensed in 100 μl containing 10^5 cells per culture well. Cultures were incubated for 1.5 hours and then washed vigorously twice to remove the nonadherent cells. Either 20 μl of medium, 20 μl of R36a vaccine containing 5×10^6 bacteria, or 20 μl of medium containing 5×10^7 SRBC were added to cultures (exposed to antigen in vitro). Cultures were incubated for 1.5 hours and then washed vigorously again. We added 100 μl of medium containing a total of 3×10^5 cells enriched for NK activity to the cultures, incubated them for 4 hours, and then washed them vigorously again. In experiment 2, A cells were prepared from spleens of mice immunized with R36a or SRBC 2 days previously and irradiated with 600 R 12 hours before they were killed (exposed to antigen in vivo). The A cells were adhered to culture wells, washed, and incubated with cells enriched for NK as in experiment 1. Plaque-forming cells, tested with either phosphorylcholine (approximately 50 PFC per culture) or SRBC (approximately 100 PFC per culture), prepared as described in Table 3, and the appropriate antigen were added to cultures. The assay times and methods for calculating the results are the same as in Table 3.

Stepwise additions to cultures				Number of PFC per culture	
1 A cells	2 Antigen	3 NK cells	4 PFC plus antigen	Against phosphoryl-choline	Against SRBC
Experiment 1: exposure to antigen in vitro					
0	0	0	+	11	28
+	+	0	+	51	184
+	+	+	+	21	52
Experiment 2: exposure to antigen in vivo					
0	0	0	+	8	9
+	+	0	+	62	100
+	+	+	+	29	43

individual's own cells that become "foreign" because of viral infection, malignant transformation (*8*), or, as found here, by acquisition of antigen by A cells. Since antigen-exposed A cells are required both for inducing and sustaining the amplified-specific immune response, early elimination or suppression of antigen-exposed A cells by NK cells would be antithetical to mounting an optimal specific immune response. While poly(I) · poly(C) induces NK activity rapidly (*7*), antigens induce NK activity later (*14*); thus, the delayed timing of NK induction by immunization may permit the early exponential increase in PFC. Poly(I) · poly(C) and probably antigens induce NK activity indirectly through induction of interferons (*3*). Presumably, the essential findings reported here should be reproducible with interferons. It is possible that the immunosuppression observed with some RNA virus infections (*15*) is mediated in part by the same mechanism that poly(I) · poly(C), a synthetic double-stranded RNA, causes suppression, that is, by induction of high levels of interferons that activate NK activity. Also, it is interesting that the aged have severe loss of inducible NK activity and dysregulation of immunity characterized by elevated serum concentrations of IgM. Our results suggest that the immune defect might result from loss of homeostatic down-regulation of antibody responses by NK cells.

In experiments to be reported separately, NK activity is inducible in irradiated mice, and the response of adoptively transferred cells is suppressed in irradiated recipients with already induced high NK activity (*16*). Thus, all of our findings taken together are consistent with the proposition that the termination of the primary antibody response of adoptively transferred cells in x-irradiated recipients is mediated by NK cells and that the same mechanism of termination may operate in the intact animal.

Intuitively, it may seem more reasonable to expect that down-regulation should be mediated by specific reactants of the response, for example, idiotypic or anti-idiotypic antibody or T cells, but one of the hallmarks of acquired immunity is that a secondary or anamnestic response can be mounted in the presence of substantial quantities of the reactants remaining from the previous response; for example, experimentally, an amount of passively given specific antibody or anti-idiotypic antibody that completely inhibits a primary response has little or no suppressive effect on secondary responses (*2*). If, indeed, responding lymphocytes in an active primary response or at the beginning of an anamnestic response are not susceptible to regulation by specific reactants, then the combination of A cell and antigen becomes an essential limiting factor and the NK system could become crucial in regulating the intensity and duration of activity of this limiting factor. Thus, our findings suggest a novel mechanism of down-regulation that permits both primary and anamnestic responses with timely down-regulation of both. The chief components are (i) induction of NK activity by immunization, (ii) a target for NK cells that is neither self (A cells) nor foreign (antigen) but rather a target that is expressed or displayed by A cells that have interacted with antigen, and (iii) a target that produces an effect or factor that is required for PFC regardless of their idiotypic specificity. Since A cells are required for many T-cell as well as B-cell reactions (*17*), it is possible that interferons and NK cells participate in regulation of specific cell-mediated immunity as well as humoral immunity. While such a mechanism of "specific immune regulation by nonspecific components" could apply generally to all immune responses, undoubtedly many other steps and mechanisms operate to regulate the magnitude and quality of immunity.

References and Notes

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5. For all cell preparations and cultures we used RPMI 1640 medium (Gibco; RPMI 1640 with 25 m*M* Hepes and L-glutamine). To this medium we added fetal calf serum, to a final concentration of 10 percent, 2-mercaptoethanol, penicillin and streptomycin, and L-glutamine. Cell preparations and cultures were incubated in 5 percent CO_2 in air at 100 percent humidity. The NK cells were the nonadherent cells prepared from spleens of 8- to 10-week-old C3H mice that received 100 μg of poly(I) · poly(C) (Sigma) intraperitoneally 24 hours before they were killed and 600 R of total body irradiation (^{127}Cs source at a rate of 200 rad/min) 12 hours before they were killed. The A cells were removed from preparations by adherence, either by two serial incubations in Falcon-3001 tissue culture dishes (*10*) or by incubation with carbonyl iron particles (*12*). Approximately 3×10^6 cells were recovered per spleen. In repeated experiments, these preparations of NK cells were added to cultures to test for their effects on PFC responses; they were also tested for cytotoxicity against YAC-1 tumor target cells by using a ^{51}Cr release assay [see J. L. Urban *et al.* (*6*)]. The assays for cytotoxicity included control cells from spleens of normal non-x-irradiated mice and cells from non-x-irradiated mice treated with poly (I) · poly (C). Assays always included multiple effector to target cell ratios (E:T) ranging from 100 or > 100:1 to 5:1. Cells from normal mice invariably caused < 50 percent lysis at an E:T of 100:1; cells from poly (I) · poly (C)–treated mice caused 50 percent lysis at an E:T of 60:1 to 30:1, while our enriched cells prepared as above always caused 50 percent lysis at E:T of < 10:1. NK activity was completely eliminated by treating spleen cells with rabbit antibody to asialo G_{M1} (Wako-Pure Chemical Industries, Ltd., Osako, Japan) and rabbit complement (Low-Tox-M, Accurate Chemical and Scientific Corp., Westbury, N.Y.). The antibody (10 μl) together with complement (100 μl/ml) was added to 10^7 cells that were then incubated for 1 hour and washed twice in fresh medium. The treatment does not affect the adherence of A cells nor the function of A cells in supporting PFC. The treatment of whole spleen cells with antiserum against asialo G_{M1} and complement does eliminate the response of cells to immunization with R36a or SRBC presumably because a target for the antiserum is shared by T cells. An antiserum to NK 1.2 (a gift from R. C. Burton), which was produced in mice [R. C. Burton and H. J. Winn, *J. Immunol.* **126**, 1985 (1981)], effectively eliminates the NK activity of cells obtained from normal mice but not from poly(I) · poly(C)–induced mice, an effect which is independent of the antibody concentration used [V. Kumar *et al.* in (*4*)]. For this reason, antiserum to asialo G_{M1}, for convenience referred to in the text as antibody to NK, was used to eliminate NK activity in the experiments reported here. The actual number of NK cells present in our preparations of cells enriched for NK cells was unknown but was presumably < 10 percent based on staining with trypan blue after treatment with the antiserum to asialo G_{M1} and rabbit complement.
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13. For the experiments reported here, A cells are the cells from spleens of mice irradiated with 600 R 12 hours previously. X-irradiation eliminates virtually all conventional B and T cells leaving about 10 percent of the normal complement of nucleated cells. NK cells are removed by treating the x-irradiated cells with antibody to asialo G_{M1} and complement (*5*), which do not affect adherence or the function of A cells in sustaining PFC. Antibody-treated irradiated cells were either added directly to cultures as indicated in the text or, in most experiments, were adhered to the bottom of culture wells (Costar 96-well tissue culture plate 3596). Cultures were incubated for 1.5 hours; the nonadherent cells were removed and wells were washed twice vigorously with 100 μl of medium. Approximately 10 percent of cells remain firmly adherent. In the experiments reported here, A cells were exposed briefly to antigen by incubating them with approximately 5×10^6 SRBC or R36a vaccine containing 5×10^7 killed bacteria in 100 μl of medium per culture for 1.5 hours. Supernatants were removed and each culture was washed twice with 100 μl of medium.
14. A syngeneic immunogenic fibrosarcoma induces in C3H mice peak NK activity at 8 days [see J. L. Urban *et al.* (*6*)]. A murine graft-versus-host reaction induced peak NK activity at 3 days [C. Roy, T. Ghayur, P. A. L. Kongshavn, W. S. Lapp, *Transplantation* **34**, 144 (1982)]. The R36a vaccine used for immunization in the experiments reported here induces peak NK activity 48 hours after immunization (L. V. Abruzzo, unpublished data).
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