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Spontaneous alloreactivity of natural killer (NK) and lymphokine-activated killer (LAK) cells from athymic rats against normal haemic cells. NK cells stimulate syngeneic but inhibit allogeneic haemopoiesis

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SUMMARY

We wanted to re-examine the hypotheses that natural killer (NK) cells preferentially react with immature cells, and that they are not directed against major histocompatibility complex (MHC) gene products. Rat marrow cells could be separated according to maturity on a four-step discontinuous density gradient of Percoll. Almost all the immature bone marrow cells with progenitor activity, as measured *in vivo* in a diffusion chamber assay or *in vitro* in a granulocyte/macrophage colony-forming assay, resided within the lighter density cell fraction (density $\approx 1{\cdot}065$). The higher density cells (density $\approx 1{\cdot}082$) contained mainly the more mature, non-proliferative cells within the granulocyte series. NK and lymphokine-activated killer (LAK) cells from athymic rats, being devoid of T cells, efficiently killed low- as well as high-density bone marrow cells from a fully allogeneic and a MHC congenic rat strain, while little or no killing was observed against syngeneic bone marrow cell fractions. LAK cells also effectively *inhibited* granulocyte/macrophage colony formation from allogeneic bone marrow precursors *in vitro*, while *stimulating* colony formation from syngeneic bone marrow cells. The NK-mediated killing of allogeneic bone marrow cells was effectively inhibited by NK-sensitive tumour cells, while there was much less inhibition of the killing of tumour cells by allogeneic bone marrow cells. We conclude that NK cells recognize MHC incompatibilities on both immature and mature allogeneic bone marrow cells through recognition systems not related to T-cell receptors, and that allospecific killing can explain the contrasting effect of NK cells on allogeneic and syngeneic haematopoiesis.

INTRODUCTION

In mice and rats histo-incompatible bone marrow cells are sometimes eliminated by a rapid, non-adaptive immune response that shows several features in common with natural killer (NK) cell function in that it requires no presensitization, is thymus independent, matures late in ontogeny, and is relatively radioresistant, but sensitive to agents that selectively disturb NK cell function.[1-4] In rats, this rejection, called *allogeneic bone marrow cell* cytotoxicity (ABC), has been studied most extensively in athymic nude animals.[5] We have previously shown that non-adherent spleen cells selectively recognize and kill allogeneic bone marrow and lymphoid cells in a short-term cytotoxic assay *in vitro*.[5-7] We have recently shown that interleukin-2 (IL-2)-stimulated spleen cells (LAK cells), that express NK cell markers but fail to express any of the T-cell specific markers or T-cell receptors, still retained their alloreactivity.[8,36] Here, we have separated the allogeneic bone marrow targets into cell fractions enriched for either immature blast cells or more mature blood cell precursors, to see if the effector cells of ABC showed any selectivity for certain cell subpopulations within the marrow. Furthermore, we have investigated the influence of LAK cells on syngeneic and allogeneic progenitor cell proliferation *in vitro*. The relationship between ABC *in vitro* and traditional NK activity against tumour cells was investigated in cross-competition studies.

Abbreviations: ABC, allogeneic bone marrow cell cytotoxicity; ADCC, antibody-dependent cellular cytotoxicity; ALC, allogeneic lymphocyte cytotoxicity; BM, bone marrow; FCS, foetal calf serum; GVH, graft-versus-host; Ig, immunoglobulins; IL-2, interleukin-2; LAK cell, lymphokine-activated killer cell; LGL, large granular lymphocyte; MHC, major histocompatibility complex; NK cell, natural killer cell; PBS, phosphate-buffered saline; RT1, the MHC locus in rats; Th, T-helper cell; TcR, T-cell receptor complex; Tc, T cytotoxic cell.

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MATERIALS AND METHODS

Animals and graft-versus-host (GVH) assay

Breeding pairs of rats from the AO ($RT1^u$), DA ($RT1^a$), PVG, PVG nude ($RT1^c$), or the PVG congenic strain PVG-$RT1^u$

(RT1^u) were a kind gift from Dr E. Bell, University of Manchester, U.K. They were reared under conventional conditions in our animal house facilities, and used at 2–4 months of age. Athymic nude rats were treated sequentially with different antibiotics (tetracyclins, erythromycin and tylosine). The GVH reactivity of lymphoid cells from PVG rats was tested in (PVG × DA)F_1 hybrid rats in the popliteal lymph node assay according to the method described by Ford & Simonsen.[9]

Generation of effector cells

Nylon-wool non-adherent spleen cells from athymic PVG rats were collected as described elsewhere.[10] In some experiments nylon-wool non-adherent spleen cells were stimulated in culture for approximately 1 week with IL-2. Human recombinant IL-2 (Amersham, Amersham, Bucks, U.K. or Cetus, Emeryville, CA) was added to the cells at 1000 IU/ml only at the beginning of culture. The culture medium was RPMI-1640 (Gibco, Paisley, Renfrewshire, U.K.) supplemented with 10% foetal calf serum (FCS) (Gibco), 2 mM glutamine, 1 mm Na pyruvate and 5×10^{-5} M 2-mercaptoethanol, hereafter referred to as LAK medium, according to Hiserodt *et al.*[11]

Separation of target bone marrow and peritoneal cells

A modification of the method described by Barlozzari *et al.* was applied.[12] Bone marrow (BM) cells were flushed out of the tibias and femurs, gently suspended and filtered once through a thin layer of cotton wool. Percoll (Pharmacia, Uppsala, Sweden) was adjusted to 285 mOsm/l with 10× phosphate-buffered saline (PBS), and RPMI + 10% FCS to 290 mOsm/l with sterile water. Osmolarity-corrected Percoll and medium were then mixed in different proportions to create four different densities: F_1 containing 37·8% Percoll (corresponding to a density relative to water of 1·053), F_2: 47·9% (1·065), F_3: 56·3% (1·075) and F_4: 62·2% (1·082). Three millilitres of each fraction were layered carefully on top of each other as depicted in Fig. 1, with 1 ml of osmolarity-corrected medium containing $30–40 \times 10^6$ BM cells at the top, in 15 ml Falcon centrifuge tubes, Cat. no. 2095, and spun at 400 ***g*** for 30 min at room temperature. The BM cells in each layer were washed out of the Percoll before being used, and are hereafter termed BM fraction-1–4 (F_1–F_4), so that, e.g. F_2 BM cells are the cells just above the second density layer. In one experiment peritoneal macrophages were isolated from rats injected i.p. on the two previous days with Bactotryptone. These cells were separated on Percoll density gradients identical to the ones used for bone marrow cell separation. F_2 cells yielded more than 90% pure macrophages.

Cytotoxicity assay

A standard NK assay was applied.[7,13] To ^{51}Cr-labelled cells, at 10^4 cells per well, were added effector cells and phytohaemagglutinin (PHA; Wellcome Diagnostics, Dartford, Kent, U.K.) at 80–100 micrograms of PHA per ml (diluted 1/100), when the target cells were BM cells. The effector and target cells were incubated for 4 hr at 37° and supernatants harvested with a Titertek Skatron (Lierbyen, Norway) harvesting system. Experimental ^{51}Cr release was calculated according to a standard formula[7]) using median values from triplicate cultures. Spontaneous release from target BM cells alone ranged from 15% to 35% of the total c.p.m. in the targets, and the total incorporation of isotope per 10^4 cells ranged from 1500 to 4000 c.p.m., the immature cells incorporating more isotope per cell than the mature cells. In the inhibition experiments, the unlabelled inbibitor cells, labelled target cells, and PHA were mixed well before the effector cells were added.

Rat progenitor cell assay

The method described by Benestad *et al.*[14] for the growth of granulocyte/macrophage colonies from mouse marrow cells was adopted for rat BM cells with the following modifications. The BM cells ($0·5 \times 10^6$), with or without LAK cells, were transferred

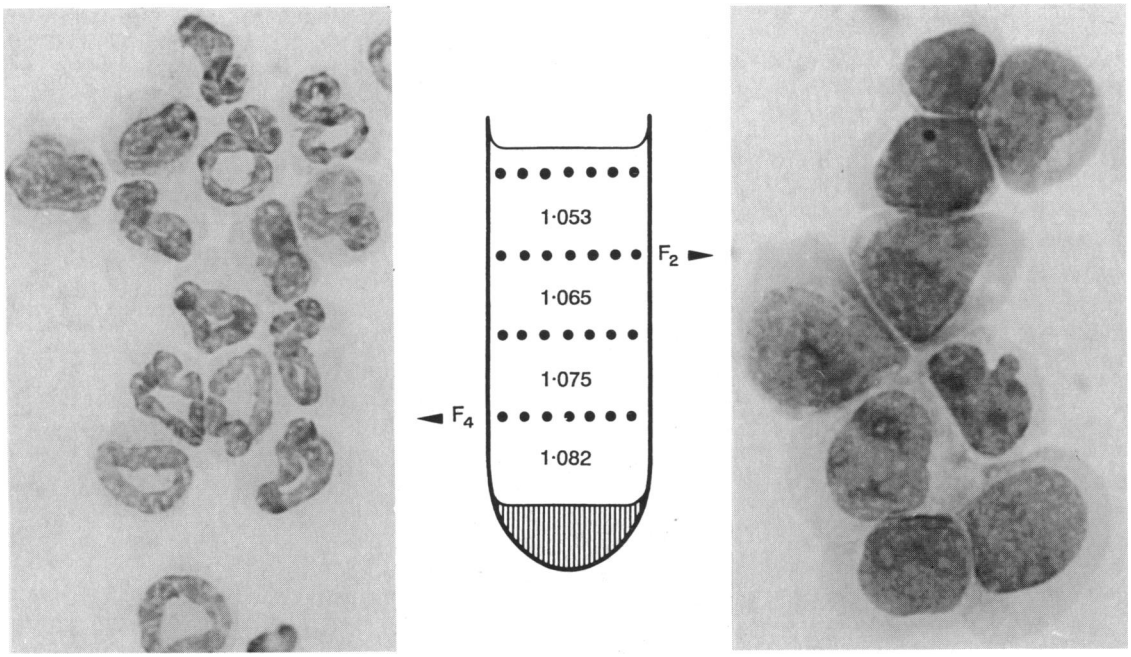

Figure 1. Giemsa-stained BM cells from Percoll separated F_2 (right) or F_4 (left) cells. The majority of F_2 cells are large immature cells with lightly stained nuclei and a basophilic cytoplasm. Among the F_4 cells non-proliferative granulocytes predominate.

to conical 50-ml Falcon centrifuge tubes in 1 ml of LAK medium, spun at 200 *g* for 7 min, and incubated at 37° in a humidified CO_2 atmosphere for 22 hr in the presence of 17% v/v conditioned medium containing colony-stimulating factors (conditioned medium from pokeweed mitogen-stimulated 7-day mouse spleen cell cultures; S-CM).[14] In other experiments it was shown that mouse spleen cells produced more potent colony-stimulating factors for rat progenitor BM cells than did rat spleen cells. The cells were then seeded with S-CM (17% v/v) and methylcellulose-containing medium at 10^5 cells per dish, and cultured for 7 days. Cell aggregates larger than approximately 50 cells were scored as colonies.

Differential counts were routinely done on coded May–Grunwald Giemsa stained smears of cells harvested from the dishes, according to morphological criteria established previously.[15] One reason for this scoring was to exclude the possibility that pseudocolonies arose from the LAK cells. Among the cells harvested were both proliferative and non-proliferative neutrophilic granulocytes, non-proliferative eosinophilic granulocytes, and macrophages. The cell composition was essentially the same in cultures from PVG and PVG-RT1^u rats, while BM cells from AO rats contained slightly less macrophages (median values 64% versus 82%) and more non-proliferative granulocytes (27% versus 13%) than PVG or PVG-RT1^u rats. No viable LAK cells could be observed.

Estimation of DNA synthesis and DNA contents of the bone marrow cell fractions

One hour before killing the animals, BM donors were given i.v. [^{3}H]thymidine (code TRK 120, Amersham) at 37 KBq (1 μCi) per gram of body weight. Samples of the various BM cell fractions were processed for scintillation counting as described elsewhere.[16] As the number of desintegrations per minute (d.p.m.) per 10^6 cells varied considerably between experiments (from 1000 to 10,000 d.p.m.), the results were normalized so that the F_2 value was set to 100%. DNA histograms were obtained after flow cytometry of propidium iodide-stained BM cells.[17] The fraction of the cells in G_1 phase of the cell cycle was determined, on the assumption that G_1 cells are contained within a histogram region ranging from G_1 top channel number −5SD to top channel number +3SD (of the G_1 peak).[18]

Diffusion chamber assay

Detailed accounts have been given.[15,19] One-hundred microlitre samples containing approximately 1×10^6 cells in RPMI-1640 with 20% v/v normal horse serum and ampicillin (200 μg per ml) (Doktacillin, Astra, Soedertaelje, Sweden) from a monodisperse suspension of BM cells, were instilled into 0·2 μm porosity chambers. Four chambers were implanted i.p. into rat hosts which had been treated with cyclophosphamide (50 mg/kg body weight i.p.; Sendoxan, Pharma, Frankfurt, Germany) on the previous day. The cells were harvested from the chambers after 4–5 days, according to a standard method.[19]

Statistics

The results were presented as medians with their 95% confidence intervals when six or more replicate observations were available, a number needed for the non-parametric estimation used. Statisitical significance was determined with a two-sided Wilcoxon's rank test, or with a modified Wilcoxon–van Elteren test for paired comparisons, applicable to independent groups of test and control samples.[20]

RESULTS

Susceptibility of Percoll-separated BM cell fractions to attack by syngeneic and allogeneic NK cells

Rat BM cells can be separated morphologically and functionally on a four-step discontinuous density gradient of Percoll.[12] By using slightly different Percoll densities, and by omitting the Isopaque–Ficoll step, we also included mature granulocytes in our fractions. The light density F_2 cells contained predominantly immature proliferative BM cells (Table 1, Fig. 1), where most of the progenitor cells for neutrophilic and eosinophilic granulocytes and macrophages were also found (Tables 2 and 3). The high cell turnover among these light density cells was disclosed both by the [^{3}H]thymidine uptake per cell, and by the proportion of cells with DNA content higher than that found in G_0/G_1 phase. These parameters were 5–10 times higher for F_2 cells than for F_4 cells (Table 1).

Among the denser cells in F_4, non-proliferative granulocytes predominated, with few immature cells (Fig. 1, Table 2), and low or absent progenitor cell activity (Tables 2 and 3). In F_3 a mixture of immature and mature granulocyte precursors and

Table 1. Cell yields, morphology, and turnover of Percoll-separated BM fractions in AO and PVG rats

	Percoll fractions					
	F_2		F_3		F_4	
[^{3}H]thymidine uptake (% of F_2)	100		65**		8**	
% of cells out of cycle	93		90		99**	
	Differential counts (%)					
	F_2		F_3		F_4	
	AO	PVG	AO	PVG	AO	PVG
Undifferentiated blast cells	31	34	8	8	2·5	3·6
Proliferative granulocytes	29	35	34	36	17	19
Non-proliferative granulocytes	2·2	1·6	7	8	59	54
Lymphocytes	26	19	25	26	7	8
Erythrocyte precursors	9	18*	25	18	11	14
Other cells	1	0	4·5	3	3·5	7
Mitoses	1·5	0·9	0·5	0	0	0
Cell yields (% of input)	6·3	3·9*	38	32	24	35*

Median values from 15 AO rats and eight PVG rats are presented.
* Statistically significant difference AO versus PVG, $P < 0.05$.
** Statistically different from F_2 values, $P < 0.05$.

Table 2. Cellular composition of BM F_2-F_4 cells from AO rats after culture in cell impermeable diffusion chambers in syngeneic hosts for 4–5 days

	Percoll fractions		
	F_2	F_3	F_4
Cytopoietic capacity†	2·38	0·74	0·50
	Differential counts‡ (%)		
Blasts + proliferative granulocytes	22	23	10*
Non-proliferative granulocytes	29	27	65*
Macrophages	41	50	25*
Lymphocytes	9	8	7
Eosinophils	0·5	2·0	1·8
Erythrocyte precursors	1·2	0·0	0·2

† Number of cells harvested from the chambers on Days 4–5 relative to the number implanted on Day 0.

‡ Median values from three experiments. Megakaryocytes were not found.

* Statistically different from F_2 values, $P < 0·05$.

Table 3. Granulocyte/macrophage colony formation *in vitro* from BM cell fractions F_2–F_4

	Number of colonies per dish	
	AO	PVG
Percoll fraction: F_2	170 (86–256)	25 (10–59)
F_3	24* (19–36)	15 (10–26)
F_4	1* (0–2)	2* (1–5)

Median values with their 95% confidence intervals from two to three experiments including a total of 12–18 dishes are given.

* Statistically different from corresponding F_2 values, $P < 0·05$.

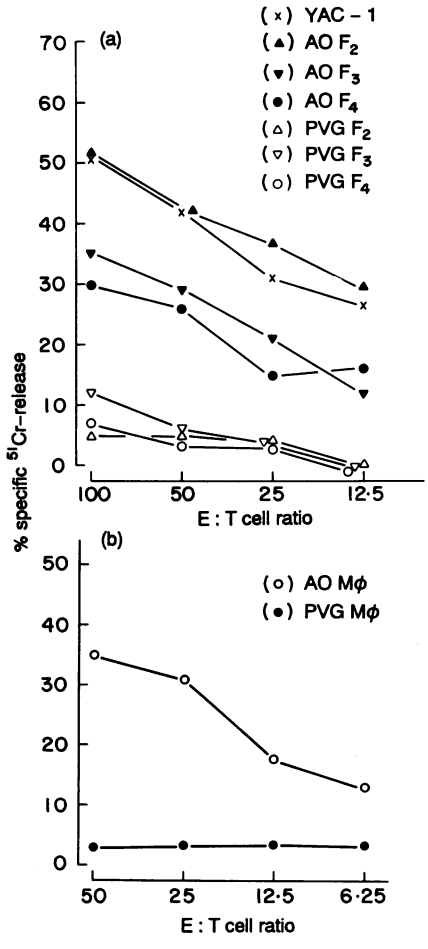

Figure 2. (a) Spontaneous NK-like alloreactivity (ABC activity) of nylon-wool non-adherent spleen cells from PVG athymic nude rats against allogeneic (AO) and syngeneic (PVG) BM cells from F_2–F_4 of a Percoll gradient centrifugation. For comparison is shown the NK activity against the rat NK-susceptible tumour cell line YAC-1. Median values of triplicates are shown from one representative experiment out of three. The killing of AO F_3 and F_4 BM cells (▼, ●) using a Wilcoxon–van Elteren test for all the data was significantly lower than the killing of YAC-1 (×) or AO F_2 BM cells (▲) ($P < 0·05$) but significantly higher than the cytotoxicity against any of the PVG BM cell fractions ($P < 0·05$). (b) NK-like alloreactivity against peritoneal macrophages.

most of the erythrocytic precursors was found (Table 1). Furthermore, this distribution of cells between the various density layers was essentially identical in the different rat strains tested (Table 1).

Nylon-wool non-adherent spleen cells from athymic PVG rats at approximately 2 months of age are almost devoid of cells with T-cell markers. Thus, less than 6% of them expressed the T-cell markers CD3, CD4, CD5, or the T-cell receptor alpha/beta (TCR2), while 51–55% of them were positive for markers present on NK cells, such as NKR-P1, CD8 and CD2).[36] In a standard 4-hr ^{51}Cr-release assay with PHA added to the BM target cells, both the low- and high-density BM cells from the allogeneic AO strain were effectively killed by nylon-wool passed spleen cells from the PVG nude strain with high NK activity against YAC-1 (Fig. 2a). However, the light density (F_2) cells were usually slightly more susceptible to lysis than the heavier cells (Fig. 2a). Only a feeble lysis of the light density cells from the syngeneic PVG strain was observed (Fig 2a). In one experiment we also tested the lysis of allogeneic versus syngeneic macrophages. Again, the allogeneic cells were preferentially killed (Fig. 2b) indicating that the NK cells discriminated between allogeneic and syngeneic leucocytes regardless of type or stage of differentiation.

LAK cells generated from spleen cells of athymic nude rats: selective cytotoxicity for allogeneic BM cells

Nylon-wool separated spleen cells from both euthymic and athymic nude PVG rats, when cultured *in vitro* in the presence of human recombinant interleukin-2 (IL-2) for approximately 1 week, generated large activated cells with prominent azurophilic granules in their cytoplasm. These cells had high NK activity

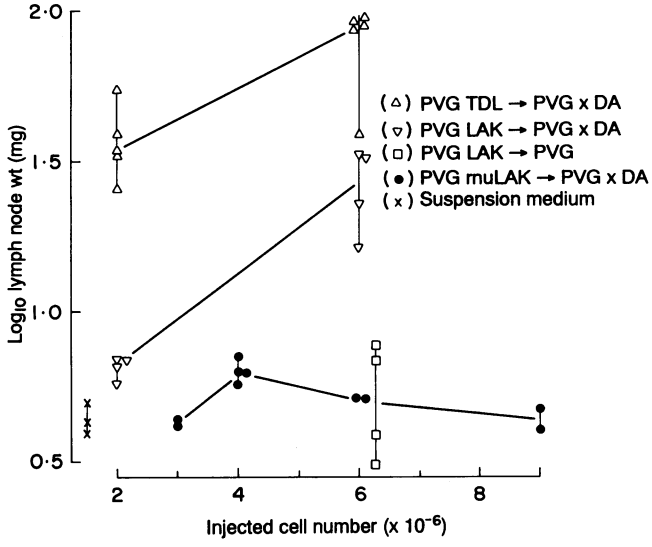

Figure 3. GVH activity of LAK cells from PVG euthymic and athymic nude rats in the popliteal lymph node assay. For comparison is shown the GVH activity of normal PVG thoracic duct lymphocytes (TDL), predominantly consisting of T cells. Values from individual lymph nodes are given, with lines connecting the median values. The GVH activity of PVG-LAK cells was significantly lower ($P < 0.05$) than that for PVG- TDL, but higher than the GVH activity of PVG-rnu LAK cells ($P < 0.05$); the latter was not different from the syngeneic control or suspension medium.

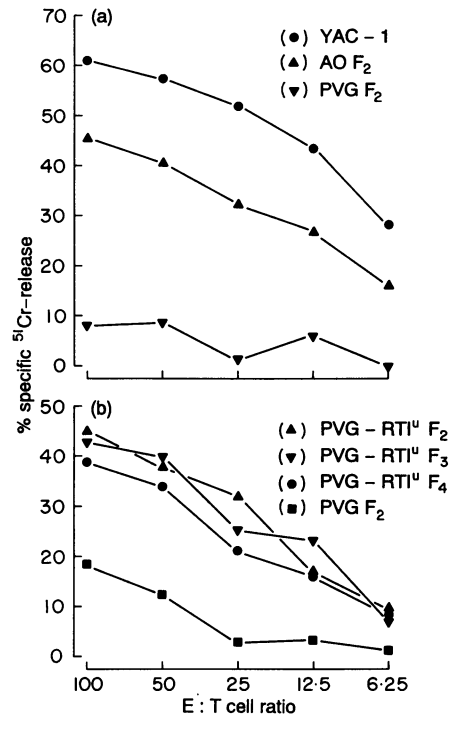

Figure 4. (a) Spontaneous NK-like allospecific cytotoxicity (ABC activity) of LAK cells generated from spleen cells of PVG athymic nude rats against fully allogeneic AO F_2 BM cells, syngeneic PVG F_2 cells, and against YAC-1 cells. (b) ABC activity of LAK cells from PVG athymic nude rats against BM cell fractions F_2–F_4 from the MHC congenic strain PVG-RT1^u compared with the cytotoxicity against syngeneic F_2 BM cells. Here, equal killing of all allogeneic BM cell fractions was observed, all being significantly ($P < 0.05$) higher than the killing of PVG F_2 cells. Killing of the latter cells was low and sometimes not detectable.

against the standard NK target YAC-1 (Fig. 3). LAK cells from athymic rats were even more devoid of T cells than the unstimulated spleen cells. Thus, functional rearrangement of the beta-chain or gamma-chain of the T cell receptors did not take place,[8] and molecules characteristic of T cells, such as CD3, CD4, CD5 were present on less than 2% of the LAK cells. However, more than 95% stained with a monoclonal antibody against the lectin-related membrane protein NKR-P1,[21] present mainly on NK cells and so far being the most specific NK cell marker in the rat.[36] Furthermore, in contrast to LAK cells generated from euthymic rats, these cells failed to display any GVH-reactivity, showing that functional T cells were not present (Fig. 3). However, PVG-nu LAK cells showed alloreactivity in that they lysed F_2–F_4 BM cells from both the AO and PVG-RT1^u strain much more effectively than syngeneic BM cells (Fig. 4a, b). Cytotoxicity against the latter cells was often hardly detectable.

Relationship between the NK-mediated killing of tumour cells and allogeneic BM cells

To further examine the relationship between the killing of tumour cells and normal allogeneic BM cells, we tested the ability of a standard tumour NK target, YAC-1, to inhibit the lysis of AO F_2 BM cells and vice versa in cold target competition studies. A clear inhibition of the bone marrow targets by YAC-1 was observed, while there was much less inhibition of the tumour cells by cold BM cells (Fig. 5).

Ability of LAK cells to selectively inhibit colony formation from MHC incompatible BM cells in viscous medium *in vitro*

Preincubation of F_2 target BM cells from AO as well as PVG-RT1^u rats with PVG-nude LAK cells at a ratio of 1:1 inhibited the subsequent granulocyte/ macrophage colony formation from the marrow cells 15–30 fold. At a ratio of 1:10 the colony formation was almost completely inhibited, at least from AO-BM (Table 4). On the contrary, a clear *stimulation* of the formation of granulocyte/macrophage colonies was observed when syngeneic PVG F_2 cells were similarly preincubated with PVG-rnu LAK cells. However, the average colony was somewhat smaller in size when LAK cells were in great excess (Table 4).

DISCUSSION

In the present study we have taken advantage of the *in vitro* test system for ABC which allows a more precise identification of the effector cells. We conclude that NK cells and the effector cells of ABC belong to the same cell population on the following premises.

(i) ABC and NK activity were both present among splenic NK cells and IL-2-activated NK cells (LAK cells) from athymic nude rats. The latter cells were completely devoid of T cells, as shown by their deficient GVH reactivity and failure to rearrange or functionally express any of the T-cell receptors or the T-cell receptor associated complex CD3.[8]

(ii) Cross-competition studies showed that NK sensitive tumour cells effectively inhibited the killing of allogeneic BM

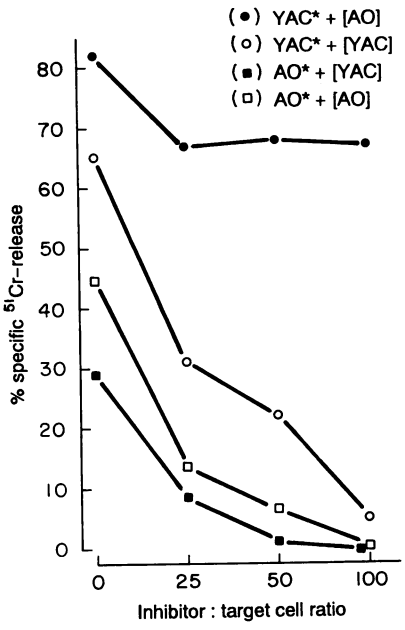

Figure 5. Reciprocal cold target inhibition of YAC-1 versus AO F_2 BM cells. Unlabelled inhibitor cells denoted in parentheses, labelled cells with an asterisk. Effector: target cell ratio was 100:1 at all inhibitor: target cell doses. One representative experiment out of three is shown. A marked cross-inhibition of killing of allogeneic BM cells with YAC-1 cells was observed, being significant already at I:T ratio 25 ($P < 0.05$). In contrast, AO BM cells only marginally, though significantly ($P < 0.05$) inhibited the killing of YAC-1 cells at all I:T ratios.

target cells. This inhibition was not due simply to steric blocking by an excess of unlabelled cells, since in the reverse combination the killing of YAC-1 was poorly inhibited by equal numbers of allogeneic BM cells. A failure of allogeneic lymphoblastoid cells to block the killing of tumour cells by allospecific NK cell lines has also recently been observed in human systems.[22]

However, it cannot be inferred from the present data that NK cells use exactly the same receptor systems for the recognition of allogeneic cells and tumour cells. May be the recognition of normal allogeneic cells requires alloantigen-specific NK receptors while the recognition of tumour cells involves multiple receptor systems that also may comprise non-polymorphic cell adhesion molecules present on all NK cells and on NK-sensitive tumour cells.[23] If receptors for different allospecificities are present on non-overlapping or only partly overlapping NK cell subpopulations as is indeed suggested by some recent data from mice and humans,[24-26] this may explain the present cross-competition data. It also gives an explanation of the fact that NK activity can occur in the absence of alloreactivity, as shown with different NK cell clones in human systems.[25]

The present experiments also give further information about the distribution of the target cell structures recognized in ABC. The idea that NK cells have a common recognition mechanism for any immature cell, which does not discriminate between allogeneic and syngeneic cells, did not find support here. Thus NK and LAK cells were poorly cytolytic against both immature and mature syngeneic BM cells, but highly cytolytic against immature as well as mature allogeneic BM cells and also

Table 4. The effect of PVG-rnu LAK cells on granulocyte/macrophage colony formation from syngeneic and allogeneic BM cells *in vitro*

Source of F_2 BM cells	Ratio between LAK cells and BM cells† 0:1	1:1	10:1
	Number of colonies per dish‡		
AO	94§ (75–116)	6¶* (5–18)	1¶* (0–3)
PVG-RT1^u	56§ (36–70)	2¶* (0–14)	8¶* (0–16)
PVG	25§ (22–27)	41††* (23–62)	67‡‡* (32–114)

† LAK cells cultured in the absence of BM cells did not generate visible colonies, but numerous cellular 'ghosts' from LAK cells could be retrieved from the dishes after a week. These cell remnants were also present in the cultures where LAK cells had been co-cultured with BM cells.

‡ Numbers give median values and 95% confidence intervals from three experiments (nine dishes) for AO and PVG-RT1^u BM cells, six experiments (18 dishes) for PVG cells.

§ These cultures contained 2·3–2·5% proliferative and 13–27%non-proliferative neutrophilic granulocytes, 2–7% non-proliferative eosinophilic granulocytes and 64–82% macrophages. While PVG and PVG-RT1^u cultures had virtually identical composition of cells, cultures from AO-BM contained slightly less macrophages and more granulocytes.

¶ These cultures contained few live cells, but mostly debris from LAK and BM cells.

†† Colonies were identical in composition to colonies from PVG BM alone.

‡‡ Colonies, although being somewhat smaller in average size than the colonies derived from BM cells alone, contained numerous viable granulocytes (3%) and macrophages (97%).

* Statistically significant difference from values in 1st column, $P < 0.05$.

allogeneic macrophages. This latter observation is consistent with recent data in the mouse.[27]

The LAK cells also discriminated between allogeneic and syngeneic BM cells in the colony-forming assay, since they inhibited colony formation *in vitro* from allogeneic BM cells, but stimulated colony formation from syngeneic cells. These latter data are in apparent contrast with the numerous reports showing that NK cells may inhibit colony formation from haematopoietic precursors in syngeneic systems,[12,28,29] experiments that have led to the idea that NK cells have a physiological control function in down-regulating haematopoiesis. This idea has, however, been countered by some reports showing no effect, or a stimulatory effect of NK cells on some of the haematopoietic progenitor cells.[30-32] Conceivably, variations in the experimental systems used, such as source of haematopoietic precursor (BM versus spleen versus blood) and stage of maturation of the progenitor cell assayed, may explain at least some of these apparently discrepant results. Although erythrocytic precursors were absent in our colony assay, this assay tested at least multipotent progenitors for all the myelogenic leucocytes (Table 4). The mechanisms responsible for the stimulation of syngeneic haematopoietic precursors can only be speculated on: NK cells are known to release a number of

cytokines including colony-stimulating factors,[33,34] that may stimulate haematopoiesis both *in vitro* and *in vivo*.

The most likely hypothesis to explain the findings in the previous and present papers is that ALC and ABC are manifestations of hitherto unidentified allorecognition systems connected to NK cells instead of T and B cells, as has also been suggested by studies with human NK cell clones.[25,26] In contrast to these reports where NK clones generated after alloantigen stimulation were examined here, we employed either freshly isolated NK cells or cells stimulated with IL-2 in the absence of antigenic stimulation, thus showing that NK cells need no previous encounter with antigen to spontaneously recognize and kill allogeneic haematopoietic cells. The fact that killing of BM cells or lymphocytes can take place between MHC-congenic rat strains indicates that the target antigens are coded for by genes located within, or closely linked to the MHC region.[35] Since these target antigens, being present probably in most haemic cells, cannot be the class II antigens, and are most likely not the class I antigens either,[35] one should search for other gene products from the MHC region that could serve as putative target antigens in ALC and ABC.

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