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Suppressive role of NK cells in pokeweed mitogen-induced immunoglobulin synthesis: effect of depletion/enrichment of Leu 11b$^+$ cells

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SUMMARY

Natural killer (NK) cells probably have immunoregulatory effects. However, the evidence to date is mainly based on the suppressive effect of enrichment with relatively impure NK populations (large granular lymphocytes, LGL, Leu 7a$^+$ cells). Here we report on the effect of enrichment and depletion of Leu 7a$^+$ and Leu 11b$^+$ cells (the latter containing virtually all NK activity in freshly prepared lymphocytes) on pokeweed mitogen (PWM)-induced immunoglobulin (Ig) synthesis. Enrichment suppressed Ig synthesis to a degree dependent on the number of cells added, and was not enhanced further by their pretreatment with interferon. Furthermore, depletion of Leu 11b$^+$ cells from peripheral blood lymphocytes (PBL) led to marked enhancement (2–25-fold increase) of Ig synthesis, suggesting these cells may normally exert a suppressive effect. The possible underlying mechanisms were investigated further. Enhanced Ig synthesis by Leu 11b-depleted cultures was associated with an increased number of Ig-secreting cells by plaque assay, but with no change in numbers of CD4$^+$ or CD8$^+$ cells. Treatment of PBL with monoclonal antibodies (anti-Leu 7a/Leu 11b) alone suppressed PWM-induced immunoglobulin synthesis. We conclude that NK cells play a role in the regulation of Ig production, at least in part by an effect on activation/differentiation of B cells, but independent of altered T-cell subpopulations. The effect may be unrelated to their cytotoxic function (being unaffected by interferon, IFN), although the direct effects of anti-Leu 11b and Leu 7a in enhancing the suppressive effect suggest an alternative activation pathway.

INTRODUCTION

Natural killer (NK) cells are defined generally by their ability to lyse various tumour or virus-infected cells in a non MHC-restricted fashion without presensitization (Trinchieri & Perussia, 1984). This dependence on an operational definition has hampered study of the role of these cells. Morphologically, large granular lymphocytes (LGL), which may be separated on density gradients, seem to contain the population of NK cells in peripheral blood, and over the past few years a variety of monoclonal antibodies have been raised to identify cells with NK activity. Anti-Leu 7a (Abo & Balch, 1981) recognizes a 110,000 MW antigen on LGL, and NK activity predominates in the Leu 7a$^+$ LGL, although Leu 7a$^-$ cells also have some activity. Anti-Leu 11b (Lanier *et al.*, 1983), which recognizes an epitope of the type II Fc receptor (CD16) present only on LGL and neutrophils, best defines cells with NK activity—virtually no NK cells being found in the Leu 11b$^-$ population. There is only a 40–50% overlap between the Leu 7a and Leu 11b markers (Lanier *et al.*, 1983).

In addition to their ability to lyse various tumour or virus-infected cells, it has been found more recently that NK cells may mediate immunoregulatory effects. Brevia, Targan & Stevens (1984) reported that LGL suppressed specific anti-tetanus toxoid immunoglobulin synthesis by lymphoblastoid B cells produced following *in vivo* tetanus toxoid immunization; the effect was enhanced by interferon (IFN, known to activate NK cells) and reduced by Leu 7a depletion. Tilden, Abo & Balch (1983) demonstrated that isolated Leu 7a$^+$ cells suppressed PWM-induced immunoglobulin synthesis and MLR-induced T-cell proliferation, although the effect was only observed after the Leu 7a$^+$ cells were treated with immunoglobulin-coated ox erythrocytes. Arai *et al.* (1983) observed that LGL suppressed *in vitro* immunoglobulin synthesis by B cells in the presence of T

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; ELISA, enzyme-linked immunosorbant assay; FACS, fluorescence-activated cell sorter; HBSS, Hanks' balanced salt solution; IFN, interferon; LGL, large granular lymphocytes; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; NK, natural killer; ORBC, ox red blood cells; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; PFC, plaque-forming cells; PWM, pokeweed mitogen.

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cells after PWM stimulation. 'NK' cells may inhibit haematopoiesis *in vitro*—probably via a soluble factor (Hansson *et al.*, 1981, 1982; Deglliantoni *et al.*, 1985)—and have been implicated in bone marrow allograft rejection (Kiessling *et al.*, 1977; Lotzova, Savary & Pollack, 1983). In all the above reports the source of NK cells has been either LGL or Leu $7a^+$ cells, although it is now known that anti-Leu 11b (CD16) more completely defines the population of NK cells.

In view of the published data we investigated the potential immunoregulatory effect of a much purer population of NK cells, eliminating any effect of contaminating T cells present in LGL and Leu $7a^+$ cells. Furthermore, if NK cells do play a role in regulation, the effect of their depletion from peripheral blood lymphocytes (PBL) prior to stimulation might generate useful and complementary data to their enrichment (the sole strategy in the above reports). This paper reports the effect of enrichment with pure FACS-sorted Leu $11b^+$ cells and of their depletion from PBL on PWM-stimulated immunoglobulin synthesis. The results suggest that Leu $11b^+$ cells suppress immunoglobulin synthesis, and possible mechanisms were investigated by studying their effect on the number of immunoglobulin-secreting cells generated and the numbers of $CD4^+/CD8^+$ T cells in the cultures.

MATERIALS AND METHODS

Lymphocyte preparation

PBL were obtained from healthy volunteers by Ficoll–Hypaque gradient centrifugation (Lymphoprep, Nyegaard, Denmark), and resuspended at 10^6/ml in RPMI-1640 (Flow Laboratories, Irving, Ayrshire) supplemented with 10% heat-inactivated fetal calf serum (FCS; Imperial Laboratories), 2 mM L-glutamine (Gibco, Paisley, Renfrewshire), penicillin 100,000 IU/litre and streptomycin 100 mg/litre (Imperial Laboratories, Andover, Hants).

Fluorescence-activated cell sorting (FACS)

PBL were incubated with optimum concentrations of anti-Leu 7a or anti-Leu 11b (Becton-Dickenson Co., Mountain View, CA) for 30 min on ice, followed by goat anti-mouse Ig FITC (Coulter Electronics, Luton, Beds). They were sorted into positive and negative fractions using an EPICS C cell sorter (Coulter Electronics), with a purity of 95%.

Depletion of cells from PBL

Complement depletion. PBL were labelled with anti-Leu 7a, anti-Leu 11b, or an irrelevant IgM class monoclonal antibody (anti-thyroid microsomal antibody, which does not stain lymphocytes) as control, as above. Labelled PBL, 10^7, were resuspended in 200 μl of supplemented RPMI and 200 μl of rabbit complement (Buxted Rabbit Co., Buxted, Surrey) added, and incubated at 37° for 45 min with 2 μg/ml DNAse (Sigma, Poole, Dorset) added during the last 10 min to prevent clumping. Control antibody-treated PBL were >99% viable.

Indirect rosette depletion. Ox red blood cells (ORBC; Tissue Culture Services, Slough), were coated with affinity-purified goat anti-mouse IgM (Cappel, W. Chester, PA): ORBC were washed five times in 0·9% saline, and 5 ml 10% ORBC added to 5 ml 0·25% trypsin (Sigma) and incubated for 30 min at 37°. The ORBC were then washed three times (0·9% saline), 0·5 ml of 0·025% soya bean trypsin inhibitor (Sigma) was added to the packed cells for 10 min at room temperature, and washed a further three times. Fifty microlitres of packed ORBC were then mixed with 50 μl goat anti-mouse IgM (1 : 2 dilution), followed by drop-wise addition of 100 μl chromic chloride (0·1 mg/ml in 0·9% saline; BDH, Poole, Dorset). The mixture was then rotated at 20° for 1 hr, washed three times in 0·9% saline, and made up to 1% in supplemented RPMI. Cells, 5×10^6, stained with anti-Leu 11b or no antibody in 250 μl RPMI-10, were added to 1 ml 1% ORBC, rotated at 4° for 20 min, spun (500 ***g*** for 5 min at 4°), and incubated on ice for a further 30 min before gently resuspending and repeating the procedure. Rosetted cells were removed over a Ficoll–Hypaque gradient. Unrosetted (interface) cells (<1% Leu 11b-positive) were resuspended at 10^6/ml in RPMI-10 after a further three washes in PBS.

Culture

Lymphocytes were cultured in 96-well round-bottomed microtitre plates (Linbro, Hamden, CT). Either 10^5 viable depleted PBL, or 10^5 PBL with 0–10^5 sorted lymphocytes, were added to each well in triplicate. Medium or optimum concentration of PWM (1 : 600 final dilution) (Gibco) were added to a final volume of 0·2 ml/well. The plates were maintained for 7–10 days at 37° in 5% CO_2 in air, centrifuged and 150 μl of supernatant were removed from each well and stored at −70° until assayed for IgG and IgM content.

IgG and IgM concentrations

These were estimated in duplicate by an enzyme-linked immunosorbant assay (ELISA). The inner 60 wells of 96-well polyvinyl ELISA plates (Nunc, Roskilde, Denmark) were coated with 75 μl/well of goat anti-human immunoglobulin (μ- or γ-chain-specific; Cappel) in carbonate-bicarbonate buffer (pH 9·6) and left for 1–3 days at 4°. Plates were washed in PBS, with 0·05% Tween 80 and samples and standards (Boehringer reference sera No. 011015II, Lewes, Sussex) in 75 μl PBS-Tween were added to wells. After incubation at 20° for 2 hr, samples were washed with PBS-Tween and 75 μl of 1/1000 dilution of mouse anti-human Ig (μ- or γ-chain-specific) alkaline phosphatase conjugate (Sigma) in PBS-Tween were added for 2 hr at 20°. After a final thorough wash, substrate (*p*-nitrophenyl phosphate; Sigma 104) in buffer (pH 9·6) was added and the optical density at 405 nm recorded on an ELISA reader (Titertek, Flow Laboratories).

Assessment of plaque-forming cells

Undepleted or depleted PBL were cultured in 96-well, round-bottomed microtitre plates at 10^5 cells per well with PWM. After 8 days supernatants were removed (for measurement of immunoglobulin) and the cells were harvested, washed twice and adjusted to $2–5 \times 10^6$ per ml. The protein A plaque assay used was modified from Gronowicz *et al.* (1976). One part protein A (Pharmacia, Uppsala, Sweden) was added to one part sheep red blood cells (SRBC; Tissue Cultures Services), which had been washed five times in normal saline. Ten parts chromic chloride ($2 \cdot 5 \times 10^{-5}$ M in normal saline) were added, and the mixture incubated at 30° for 60 min. SRBC were then washed once in normal saline and twice more in Hanks' balanced salt solution (HBSS; Flow Laboratories) and made up to a 20% suspension in HBSS. Agar (0·5%; Difco, Detroit, MI) supplemented with 0·45 mg/ml DEAE dextran (Pharmacia) was kept at 46° in a water bath and 800 μl were aliquoted into tubes. To these were added in turn 25 μl developing antiserum (rabbit anti-human, μ- or γ-specific; Dako, High Wycombe, Bucks), diluted

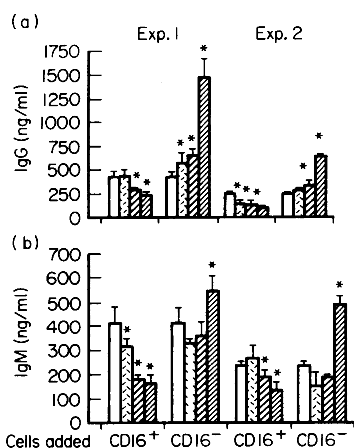

Figure 1. Enrichment with Leu 11b⁺ cells suppresses and with Leu 11b⁻ cells enhances immunoglobulin synthesis of PWM-stimulated cultures. Results of two experiments; 10^5 PBL, alone (□) or with (▧) $2{\cdot}5\times10^4$, (▨) 5×10^4 or (■) 10^5 Leu 11b⁺ or ⁻ cells as indicated, were cultured with PWM and supernatants assayed for (a) IgG and (b) IgM. Error bars represent standard deviations. * $P<0{\cdot}05$ compared with control (no added cells).

1:30 in HBSS, 50 μl protein A-treated SRBC, 100 μl of cell suspension and 25 μl guinea-pig complement (absorbed 1:1 with SRBC for 2 hr on ice, and diluted 1:2 in HBSS). The mixture was vortexed and four 100-μl drops were placed on a 90 × 15 mm petri-dish (Sterilin, Feltham) and covered with 22 × 22 mm coverslips. The plates were incubated overnight (37°, 5% CO_2, 100% humidity) and plaques were counted using indirect light. Controls included SRBC not treated with protein A, which failed to produce plaques. Results are expressed as number of plaques per million viable cells in the assay.

RESULTS

Preparation of sorted cells

Depending on the donor, 7–15% of PBL were Leu 7a⁺ and 7–12% were Leu 11b⁺ on FACS analysis. Purity of sorted populations was always >95% (and usually >98%) as assessed directly by fluorescence microscopy, FACS reanalysis or immunoperoxidase staining of cytopreps. Leu 7a⁺ and Leu 11b⁺ cells were mainly large granular lymphocytes on Giemsa staining, while the negatively sorted populations were similar to PBL and included both lymphocytes and monocytes.

Effect on PWM-stimulated immunoglobulin synthesis of enriching PBL with FACS-sorted populations of cells

Addition of increasing numbers (up to 10^5) of Leu 11b⁺ cells to 10^5 PBL resulted in a progressive and reproducible (in five separate experiments) suppression of PWM-induced IgG production, while increasing numbers of Leu 11b⁻ cells resulted in enhanced immunoglobulin production (Fig. 1a). Similar percentage changes were observed on IgM production (Fig. 1b; Table 1). Addition of increasing numbers of Leu 7a⁺ cells to PWM-stimulated PBL resulted in an increase in IgG and IgM synthesis in four experiments and a decrease in two experiments (Table 1).

Table 1. Effect of enriching cell types on PWM-induced Ig synthesis

Cell phenotypes enriched	Effect on PWM-induced Ig synthesis*			
	Enhanced		Depressed	
	No. exp.	Range (%)†	No. exp.	Range (%)
Leu 11b⁺	0	—	5	54–88
Leu 11b⁻	5	105–500	0	—
Leu 7a⁺	4	123–450	2	4–68
Leu 7a⁻	5	114–1600	1	51

* Magnitude of effect was dependent on cell number added.
† Range of maximal effects (with 10^5 sorted cells added to cultures) and compared with no added cell controls.

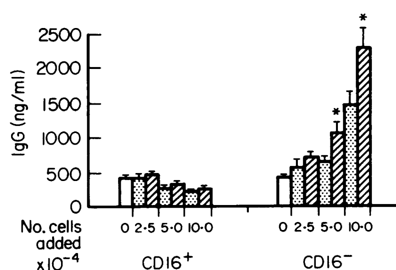

Figure 2. IFN-γ pretreatment of sorted cells (■) potentiates the effects of CD16⁻ but not CD16⁺ cells on PWM-stimulated Ig synthesis compared with untreated sorted cells (▩). Error bars represent standard deviations. * $P<0{\cdot}05$ comparing treated with untreated cells.

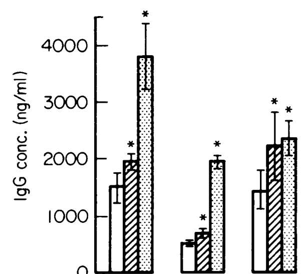

Figure 3. Depletion of Leu 7a⁺ and Leu 11b⁺ cells by complement lysis enhances PWM-induced IgG synthesis. Results of three representative experiments. (□) control, (■) Leu 7a depleted and (▩) Leu 11b depleted. Error bars indicate standard deviations. * $P<0{\cdot}05$ compared with control cultures.

In two experiments, sorted cells were incubated overnight in RPMI-10 with or without recombinant IFN-γ 1000 U/ml, before being washed and added to PBL. In separate experiments it was established that pretreatment of sorted cells with IFN-γ overnight enhanced cytotoxicity towards a NK-sensitive target (data not shown). IFN-γ pretreatment of Leu 11b⁺ cells did not result in any significant increase of suppression of IgG or IgM production, while cultures supplemented with IFN-treated Leu 11b⁻ cells consistently produced *more* immunoglobulin than non-IFN-γ-treated Leu 11b⁻ cells (Fig. 2).

Table 2. Effect of Leu 7^+/Leu 11^+ depletion on PWM-induced Ig synthesis*

	Enhancement of PWM-induced Ig synthesis†	
Depleted cell phenotype	No. experiments	Range (%)‡
By complement lysis		
Leu 7	8/9	116–5052
Leu 11	13/14	158–2515
By indirect rosetting		
Leu 11	2/3	422–1246

* 10^5 PBL or PBL depleted of Leu $7a^+$ or Leu $11b^+$ cells were cultured for 1 week with PWM.

† Immunoglobulin synthesis as assessed by IgG and IgM in supernatant. Enhancement was observed in all experiments performed, the numerator indicates the number in which this was significant at $P<0{\cdot}05$ level, compared with control cultures treated with irrelevant antibody and complement or ORBC.

‡ Percentage range of enhancement compared with control cultures.

Effect of depletion of Leu $11b^+$/Leu $7a^+$ cells from PBL

PBL were depleted of Leu $11b^+$ or Leu $7a^+$ cells by complement lysis or indirect rosetting. Depletion resulted in enhancement of PWM-induced immunoglobulin (IgG and IgM) production (Fig. 3). The enhancement occurred in all of 14 complement-depletion and three indirect rosette-depletion experiments, but did not achieve significant levels in one experiment of each type on a single subject. The enhancement was often very marked (range 116–5052% for Leu 7a depletion and 158–2519% for Leu 11b depletion; Table 2). When both Leu 7a and Leu 11b populations were depleted from separate PBL samples from the same donor, Leu 11b depletion resulted in greater enhancement than Leu 7a depletion (Fig. 3).

Effect of binding of monoclonal antibodies

In order to determine whether monoclonal antibody binding was sufficient to suppress PWM-induced Ig synthesis, PBL were incubated for 30 min with either anti-Leu 11b or anti-Leu 7a, washed and then cultured with PWM. Immunoglobulin production was significantly reduced compared with control PBL treated with an irrelevant antibody. The effect was not due to the small amounts of azide used as preservative with the monoclonals, since similar concentrations of azide alone had no effect (data not shown).

Reverse haemolytic plaque assay

Control and Leu $11b^+$ depleted PBL cultures were compared for the number of plaque-forming cells (PFC) per 10^6 cells. Figure 4 shows the results from three experiments. The depleted cultures contained more IgG (and IgM) PFC, in parallel with the increased IgG and IgM concentrations in the culture supernatants.

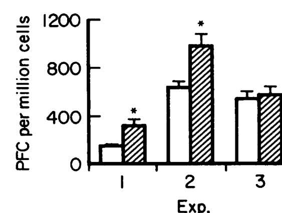

Figure 4. Depletion of Leu $11b^+$ cells results in increased PFC 7 days after PWM stimulation. Method as in the Materials and Methods. (□) Control or (■) Leu 11b-depleted cultures. Error bars indicate standard deviation. * $P<0{\cdot}05$ compared with control cultures.

Table 3. Percentage of cells $CD4^+$/$CD8^+$ after 7 days culture with PWM, with or without $CD16^+$ depletion

	PBL	Exp. 1*		Exp. 2		Exp. 3	
	(no PWM)	CTL†	$CD16^-$‡	CTL	$CD16^-$	CTL	$CD16^-$
$CD4^+$	29·9	44·0	49·7	36·2	38·8	43·7	39·4
$CD8^+$	22·2	23·7	18·1	41·0	42·8	33·1	37·7

* Cells were phenotyped after 7 days culture with PWM.

† CTL, control cultures treated with irrelevant monoclonal and complement.

‡ Depleted with Leu 11b+complement, as in the Materials and Methods.

Surface phenotype of lymphocytes

The percentages of $CD8^+$ and $CD4^+$ cells were compared in control and Leu 11b-depleted PWM cultures after 7 days. There were no major differences in percentages or numbers (Table 3).

DISCUSSION

In these studies we have shown that cells bearing NK markers play a role in the regulation of Ig synthesis following PWM stimulation. Pure populations of Leu $11b^+$ and (less consistently) Leu $7a^+$ cells, when added to autologous PWM-stimulated PBL, suppressed immunoglobulin synthesis. The effect was not enhanced by pre-incubation of NK cells with interferon (although this greatly enhances cytotoxicity by NK cells). Furthermore, marked enhancement of immunoglobulin production was seen (up to fifty times control cultures) following depletion of Leu $7a^+$ or Leu $11b^+$ from PBL, despite these cells constituting only a minor subpopulation (7–15%) of PBL.

FACS-sorted Leu $11b^+$ lymphocytes exerted a suppressive effect on PWM-induced immunoglobulin production by PBL. The magnitude of suppression was dependent on the number of Leu 11b-positive cells, but the number of such cells required was large. Addition of Leu $7a^+$ cells had a variable effect (suppression in two individuals and enhancement of immunoglobulin production in four individuals).

Enriching PBL with NK-depleted cells (FACS-sorted Leu $11b^-$ and Leu $7a^-$ populations) enhanced IgG and IgM production of PWM cultures. Although in some experiments

the increase could have been consistent with the additional B cells, in others the increase was far greater (up to 16 times control cultures with no added cells, Table 1). Furthermore, this enhancement was observed even in the presence of Leu 7a$^+$/Leu 11b$^+$ cells in the final culture (from the co-cultured unfractionated PBL), when the overall depletion could not have been more than 50%. One possible explanation is that Leu 11b$^+$/Leu 7a$^+$ cells exert an immunoregulatory role in PBL responses to PWM. Alternative explanations include altered ratios of lymphocytes to monocytes (known to affect PWM response), or an effect of the sorting/staining procedures. To exclude the latter possibilities, the effect of direct depletion from PBL of Leu 11b- or Leu 7a-positive cells was investigated and enhancement of PWM-stimulated immunoglobulin production was again consistently observed.

In contrast to previous studies we used anti-Leu 11b to select NK cells rather than the less specific density gradient separated LGL (Brevia *et al.*, 1984; Arai *et al.*, 1983) or Leu 7a$^+$ cells (Tilden *et al.*, 1983), which both exhibit a heterogeneous surface phenotype, with T-cell contamination (Ortaldo *et al.*, 1981; Krensky *et al.*, 1985; Lanier & Phillips, 1985). NK enrichment by these other methods exerts a suppressive effect on immunoglobulin synthesis in a variety of experimental systems. However, the suppression by Leu 7a$^+$ cells reported by Tilden *et al.* (1983) required 'activation' with IgG-coated ORBC and the suppression of specific anti-tetanus toxoid antibody production was seen with LGL only in the absence of T cells (Arai *et al.*, 1983). We found the suppressive effects of Leu 7a$^+$ cell enrichment to be variable and of Leu 7a$^+$ cell depletion to be less marked than with Leu 11b$^+$ depletion.

The possible mechanisms underlying this suppressive effect were investigated further. Depletion of Leu 11b$^+$ or Leu 7a$^+$ PBL resulted in a relative increase in the number of Ig-secreting cells by plaque assay. CD8$^+$ cells are suppressive to PWM-induced Ig synthesis by inhibiting B-cell differentiation (Thomas *et al.*, 1980, 1981) and depletion results in an increased number of CD4$^+$ cells (Puck & Rich 1984) and immunoglobulin production (Weetman *et al.*, 1985) in such cultures. A direct effect of Leu 11b$^+$ depletion from PBL on CD4$^+$ and CD8$^+$ T-cell numbers was not observed either at the outset or after 7 days culture in these experiments (Table 3).

There was no increase in suppression following pre-incubation of NK marker-bearing cells with IFN-γ in a dose that markedly enhanced cytotoxicity. This may suggest that the suppressive effect is unrelated to their cytotoxic potential and may depend not on lysis but on lymphokine production. It is also possible that NK cells are maximally stimulated in the PWM experimental system. However, addition of the monoclonal antibodies alone did enhance the suppressive effect, suggesting that 'activation' of NK cells by an IFN-independent pathway may be important in the suppression of Ig synthesis. It is interesting to note that Tilden *et al.* (1983) reported that Leu 7a$^+$ PBL only suppressed Ig synthesis after 'activation' with Ig-coated ORBC, presumably via Fc receptor interaction. Anti-Leu 11b (an IgM monoclonal antibody) may similarly crosslink such receptors on NK cells.

In contrast, pre-incubation of Leu 11b$^-$/Leu 7a$^-$ cells with IFN-γ resulted in greater enhancement of Ig production than without IFN treatment. This probably represents the effect of IFN-γ on PWM-induced immunoglobulin synthesis by PBL. IFN-γ pretreatment of PBL alone enhanced PWM-induced Ig synthesis (data not shown). Furthermore, others have found IFN-γ enhances B-cell responses. Leibson *et al.* (1984) demonstrated that IFN-γ can substitute for late-acting helper factor and acted synergistically with other factors in the stimulation of B-cell antibody production *in vitro*. Mond & Brunswick (1987) described experiments suggesting IFN-γ was an essential lymphokine for T-dependent B-cell responses—PWM B-cell response is T dependent.

The increased PFC observed following NK depletion suggests regulation, at least in part, at a stage before B-cell differentiation and secretion. However, an additional effect at later stages cannot be excluded. This is in agreement with other authors, including Arai *et al.* (1983) who demonstrated that LGL suppressed PWM-stimulated immunoglobulin secretion in the presence of T4 cells but not with T-cell factors alone—suggesting the effect was T-helper cell dependent. By contrast, others have presented data to suggest NK cells may act on B cells directly. Brevia *et al.* (1984) reported that LGL are able to inhibit spontaneous specific anti-tetanus toxoid immunoglobulin production by purified B cells from individuals following vaccination. This effect was enhanced by IFN and reduced by depleting the LGL of Leu 7a$^+$ cells, and following further experiments this group has suggested that a lytic process is involved in the suppression, although their evidence for this is very indirect (Targen *et al.*, 1985). More recently, Kuwano *et al.* (1986) have reported that LGL will suppress immunoglobulin synthesis by autologous Epstein–Barr virus-infected B cells. This effect was dependent on NK cells and their production of IFN-γ (which activated the NK cells but alone had no direct effect), and was abolished by Leu 11b depletion of LGL. Unfortunately, the number of immunoglobulin-secreting cells was not determined, and therefore it is not clear whether the LGL affected the number of Ig-producing cells or suppressed Ig production by immortalized cells. Certainly the outgrowth of EBV-infected lymphocytes is inhibited by non-adherent E-rosette-positive IgG–Fc receptor-positive cells and LGL (Shope & Kaplan, 1979; Masucci *et al.*, 1983), so the NK effect seen in this system may well represent NK activity against EBV. Furthermore, it is quite possible that NK cells act at several different sites; studies in mice have suggested multiple sites of potential interaction, including an effect on accessory cells (Abruzzo & Rowley, 1983).

Our own and the reports discussed above have suggested NK cells inhibit immunoglobulin synthesis *in vitro*. However, there have been studies to suggest NK cells may secrete factors stimulating B-cell growth and differentiation under certain conditions. Pistoia *et al.* (1985) and Vyakarnam *et al.* (1985) and others have demonstrated lymphokines from LGL may stimulate growth/differentiation of activated B cells. Brenner *et al.* (1986, 1987) studied recipients of T-cell-depleted bone marrow transplants. LGL from these patients spontaneously secreted IL-2, IFN-γ and B-cell differentiation factor. There was also a possibility that subpopulations of LGL with helper or cytotoxic function may exist in these patients. The different findings undoubtedly resulted from the different experimental conditions. How these relate to the normal immunoregulatory interactions in healthy individuals cannot as yet be determined.

In conclusion, our studies provide further support for the idea that cells having 'NK' phenotype and activity may exert important immunoregulatory functions. Indeed, even in 'physiological' numbers their suppressive effects are marked and

may be at least as important as suppressive effects produced by T cells, despite the relatively small number of Leu $11b^+$ cells present in PBL. The mechanisms underlying the phenomenon remain unknown, although a direct reduction in numbers of Ig-secreting cells, and a possible IFN-independent 'activation' of NK cells, may be involved. Studies to delineate further the nature of this suppression, which may involve multiple sites, should include experiments designed to more definitely exclude a direct cytotoxic effect on B cells, and to examine the role of soluble factors known to affect immunoglobulin production (such as IL-2, IL-4, IFN-γ, and neuroleukin).

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