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Endogenous and interferon-augmented natural killer cell activity of human peripheral blood mononuclear cells *in vitro*. Studies of patients with multiple sclerosis, systemic lupus erythematosus or rheumatoid arthritis

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SUMMARY

Peripheral blood mononuclear cells (PBMC) of normal human donors are spontaneously cytotoxic for certain tumour-derived and virus-infected target cells. This so-called natural killing (NK) can be augmented by the action of interferons (IFN) and by IFN-inducers. In this study, we have compared both endogenous and augmented NK activity of normal donors with that of patients suffering from either multiple sclerosis (MS), systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). Endogenous NK was assayed using an NK susceptible target cell (K562), and augmented NK using a target cell (WI-L2) which is lysed only by NK effector cells that have been pre-stimulated by IFN or IFN-inducers. While NK function appeared normal in RA patients, this study confirms previous reports of defective endogenous NK in many MS and SLE patients. In addition, anomalous IFN-augmented NK was also detected in many patients with these two diseases, indicating that defective NK function cannot always be corrected by IFN treatment *in vitro*. Analysis of IFN production, endogenous NK and IFN-augmented NK by individual patients with MS or SLE showed the defects in their IFN-NK systems to be highly selective, suggesting that individual components of this system may operate independently.

INTRODUCTION

Peripheral blood mononuclear cells (PBMC) of patients suffering from various chronic diseases of known or suspected immunological involvement function abnormally in *in vitro* tests of immune responsiveness. We have recently observed that PBMC of many patients with multiple sclerosis (MS) or systemic lupus erythematosus (SLE), but not rheumatoid arthritis (RA), produce decreased amounts of interferon (IFN) in response to a variety of IFN inducers (Neighbour & Bloom, 1979; Neighbour, Miller & Bloom, 1981; Neighbour & Grayzel, 1981). Apart from its anti-viral activity, IFN regulates a variety of immune responses including the spontaneous cytotoxicity mediated by natural killer (NK) cells (Herberman, 1980). While the nature of NK cells and their mechanism of cell lysis have yet to be determined, it has been suggested that these cells, together with IFN, play an active role in natural resistance and recovery from tumours and virus-infected cells (Herberman, 1980). The existence of an IFN-NK 'system' has been demonstrated by the observations that (a) selective enrichment for cells capable of NK also copurifies the

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cells which produce IFN in response to various inducing agents, including the tumour target cell itself; and (b) IFN and IFN inducers augment NK both *in vivo* and *in vitro*.

In this paper we have compared both endogenous and augmented NK of PBMC from MS, SLE and RA patients with normal donors. Endogenous NK was tested with unstimulated PBMC on an NK susceptible target cell (K562); and augmented NK using either IFN- or *Newcastle disease* virus-stimulated PBMC on a target cell (WI-L2) which is lysed only by NK effector cells that have been pre-stimulated by IFN or IFN-inducers.

MATERIALS AND METHODS

Patients and normal donors. Thirty-three MS patients, 18 women and 15 men with a mean age of 39 years were tested. All had clinically definite MS according to the criteria of Schumacher *et al.* (1965). Patients were categorized into one of the following clinical states: exacerbation (five patients with clear development of new neurological abnormalities or worsening of previously existing signs, lasting longer than 24 hr), remission (nine patients with clearly episodic disease who were not in a period of changing neurological status), chronic progressive (10 patients whose neurological condition was continuing to deteriorate without clearly definable exacerbations) and stability (nine patients who had previously entered a progressive stage of the disease but had shown no change in neurological examination for at least 6 months). Patients were also evaluated for severity of disease according to the Kurtzke disability status scale (Kurtzke, 1970). None of the MS patients were receiving steroids or other immunosuppressive therapy at the time of testing.

Thirty-four SLE patients, 31 women and three men with a mean age of 34 years, were tested. All patients met at least four of the American Rheumatism Association (ARA) criteria for the diagnosis of SLE (Cohen *et al.*, 1971). Thirteen patients were considered to have active disease manifested by fever, arthritis, serositis or an active urinary sediment while five patients, who were hospitalized for acute CNS symptoms, progressive renal disease or thrombocytopenia, were considered to have acute disease. The remaining 14 patients whose symptoms were limited to rash, arthralgias, Raynaud's penomenon or persistent proteinuria, were considered inactive. Patients received either no therapy ($n=4$) or one of the following: less than 20 mg/day of prednisone ($n=13$); 20 mg/day or more of prednisone ($n=10$); or a combination of prednisone and azathioprine ($n=7$).

Twenty-two RA patients, 20 women and two men with a mean age of 54 years, were tested. All patients met the ARA criteria for definite or classical RA (Ropes *et al.*, 1958) and all had a positive test for rheumatoid factor in their serum. Sixteen patients with two or more objectively warm, tender, swollen joints were considered to have active disease. The remaining six patients were considered inactive. Patients were receiving the following treatment either singly or in combination: gold salts, a non-steroidal anti-inflammatory drug, prednisone (15 mg/day or less), cyclophosphamide, or azathioprine. Fourteen of the patients were receiving steroids.

Thirty-four volunteer medical school and laboratory personnel, 19 women and 15 men with a mean age of 32 years, were tested. None of these normal donors showed any apparent signs of clinical disease nor were receiving any form of medication at the time of testing.

Effector cells. Mononuclear cells were separated from heparinized venous blood by Ficoll-Paque (Pharmacia) density gradient centrifugation. Cells were incubated for 16 hr at 37°C in a humidified atmosphere of 5% CO_2 in air at a concentration of 5×10^6/ml in medium alone (RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100 μg/ml penicillin, and 100 μg/ml streptomycin) or in medium containing either 10 μl/ml of a stock solution of beta-propiolactone-inactivated *Newcastle disease* virus (NDV; 5×10^8 pfu/ml before inactivation) or 1,000 units of partially purified human IFN-α per ml (sp. act. of $2{\cdot}5 \times 10^6$ units/mg of protein, generously provided by Dr Kari Cantell, Finland). Preliminary experiments indicated that overnight incubation of untreated effector cells did not significantly affect the level of cytotoxicity and could, therefore, be considered to represent endogenous NK. In addition, treatment with NDV or IFN under the described conditions produced optimal augmentation of NK in normal donors. After incubation, the cells were washed twice, and the number of viable cells determined by trypan blue exclusion in a haemacytometer.

Target cells. K562 cells, a human erythroleukaemia cell line, and WI-L2, a human B lymphoblastoid cell line, were maintained in continuous suspension culture in RPMI 1640 medium supplemented with 10% and 20% FCS respectively. Approximately 2×10^6 cells in 0·2 ml of medium were labelled with 100 μCi of ^{51}Cr (Sodium salt, sp. act. 350–600 mCi/mmole, Amersham) for 1 hr, washed four times and resuspended in medium at 1×10^5 cells/ml.

Cytotoxicity assay. Triplicate cultures of 1×10^4 target cells in 100 μl of medium were mixed with 100 μl of each effector cell suspension to give effector: target ratios of 80, 40, 20, and 10:1 in the wells of round-bottomed microtitre plates (Linbro). The plates were contrifuged for 5 min at 100 ***g***, and incubated for 5 hr at 37°C in a humidified atmosphere of 5% CO_2 in air. Supernates were harvested using a Titertek Supernatant Collection System (Flow Labs.) and counted in a gamma counter.

Percentage specific cytotoxicity was calculated as:

$$\text{cytotoxicity } (\%) = \frac{E - S}{M - S} \times 100$$

where E was the experimental release in counts per minute (c.p.m.) from each effector:target combination; S was the spontaneous release from 1×10^4 target cells incubated for 5 hr without effector cells; and M was the maximum release as determined by the total incorporated ^{51}Cr in an equal number of labelled target cells.

Cytotoxic activity was then determined for each donor sample by linear regression analysis of percentage cytotoxicity against effector:target ratio and expressed as lytic units (LU) per 10^7 PBMC, where 1 LU was defined as the number of effectors required to produce 30% specific cytotoxicity of 1×10^4 target cells. Cytotoxic activities of < 1 LU/10^7 cells were arbitrarily assigned the value of 0·00 $\log_{10}$ LU because solution of the linear regression equation at such low levels of lysis required excessive extrapolation and, consequently, provided a highly inaccurate estimate of lytic units. The group mean endogenous or augmented NK were calculated as the geometric mean ($\pm$s.d.) cytotoxicity by untreated or by NDV- and IFN-treated effector cells respectively. NK+ donors were defined as those whose level of cytotoxicity was above the lower 95% confidence limit for the normal group. Responders were those donors whose treated effector cells showed an increase in cytotoxicity over their untreated control effectors.

Interferon induction and titration. Induction of interferon by NDV in PBMC of normal, MS, SLE and RA donors and its titration were as reported previously (Neighbour *et al.*, 1981; Neighbour & Grayzel, 1981).

Statistics. Group NK activity was calculated as the geometric mean ($\pm$s.d.) LU and compared between groups by the Student's *t*-test after $\log_{10}$-transformation. Levels of NK augmentation were compared by a paired *t*-test, each donor's untreated effector cells serving as their own controls for NDV- or IFN-treated cells. The proportions of responders to the augmentation stimuli were compared by the χ^2 test. The influence of disease activity and therapy on endogenous and augmented NK was tested by a single factor analysis of variance. Correlation between IFN production, endogenous or augmented NK was tested by bivariate regression analysis. Significance was accepted throughout at the $P < 0.05$ level.

RESULTS

K562 cells were highly susceptible to lysis by untreated effector cells and stimulation of the effectors with NDV or IFN produced only a slight increase in the amount of lysis (Fig. 1). In contrast, WI-L2 was very resistant to endogenous NK, but was readily killed by stimulated effector cells. Consequently, K562 cells were used to measure endogenous NK, and WI-L2 cells as sensitive indicators of IFN-dependent augmented NK.

Endogenous natural killing of K562 target cells by patients' cells

The endogenous NK activities of normal and patient donors were bimodally distributed with donors exhibiting either significant killing or no killing of these target cells (Fig. 2). While the mean endogenous NK for the MS and SLE patient groups differed significantly from the normal group

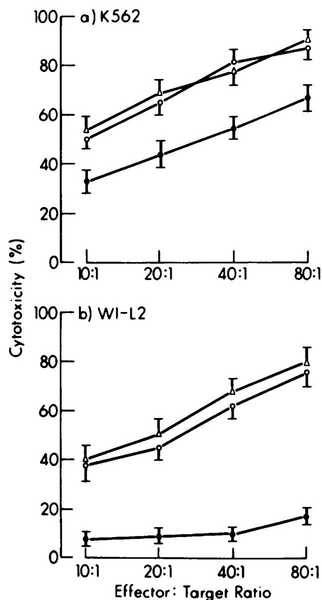

Fig. 1. Endogenous and augmented natural killing of (a) K562 and (b) WI-L2 target cells in a 5 hr ^{51}Cr-release assay by effector cells pre-incubated alone (●), with NDV (△), or with IFN (○). Cytotoxicity presented as mean ± s.d. percent specific ^{51}Cr-release from five donors.

(Table 1), the bimodal distribution of data prevented valid comparison of group means. Therefore, the 95% confidence limit derived from the normal group data (Fig. 2) was used to separate individuals as either NK+ or NK− donors. Table 1 shows that there were significantly fewer NK+ donors in the MS and SLE patient groups. However, while the mean endogenous NK for the NK+ donors in each of these two patient groups was consistently lower than in the appropriate normal group, the differences were not significant. In contrast, while the RA patients exhibited

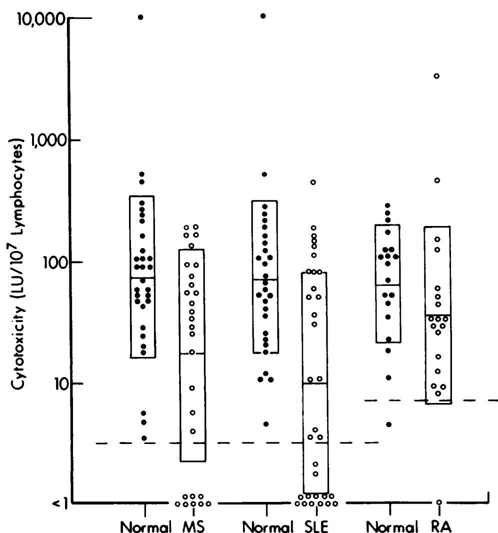

Fig. 2. Distribution of endogenous natural killing of K562 target cells by PBMC from normal, MS, SLE and RA donors. Dotted line represents the lower 95% confidence limit for the normal group.

Table 1. Endogenous natural killing of K562 target cells by MS, SLE and RA patient's lymphocytes *in vitro*

		All donors		NK positive donors*		
		Cytotoxicity			Cytotoxicity	
Group	No.	($\log_{10}$ LU)†	(LU)	Proportion (%)	($\log_{10}$ LU)†	(LU)
Normal	30	1·882±0·67	76·2	100·0	1·882±0·67	76·2
MS	29	1·224±0·87‡	16·7	72·4‡	1·690±0·49	49·0
Normal	28	1·850±0·65	70·8	100·0	1·850±0·65	70·8
SLE	32	0·991±0·93§	9·8	59·0§	1·639±0·62	43·5
Normal	19	1·809±0·48	64·4	94·7	1·872±0·40	74·4
RA	19	1·547±0·73	35·2	94·7	1·633±0·65	42·9

*Donors whose NK did not differ significantly from the normal group mean cytotoxicity ($P<0·05$).
†Geometric mean ± s.d.
‡Significantly different from normal group: $P<0·01$.
§Significantly different from normal group: $P<0·001$.

slightly lower endogenous NK than normal group, differences in mean cytotoxicity and in proportion of NK+ donors did not differ significantly.

Augmented natural killing of K562 and WI-L2 cells

Although there was no significant difference in the proportion of MS patients showing

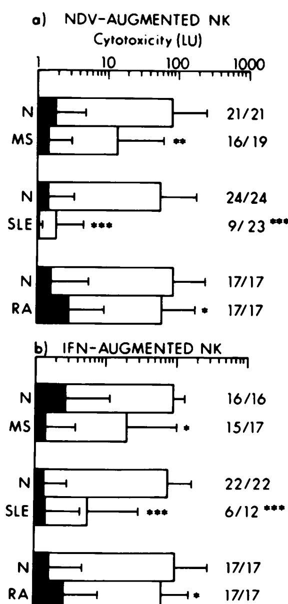

Fig. 3. Augmented natural killing of WI-L2 target cells by (a) NDV-treated and (b) IFN-treated PBMC from normal (N), MS, SLE and RA donors. Data represented as the mean ± s.d. cytotoxicity (LU/10^7 cells) by control effectors (solid bars) or augmented effectors (open bars). The numbers of responders out of the numbers of donors tested in each group are also indicated. Significant differences from normal group: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

augmentation of NK by NDV or IFN on WI-L2 cells, the levels of augmentation were significantly reduced (Fig. 3). There was no significant difference between the normal and MS patient groups when augmented NK was tested on K562 targets (data not shown). In contrast to the MS patient donors, the SLE patients showed a highly significant defect in their response to augmentation by NDV or IFN on both WI-L2 (Fig. 3) and K562 (data not shown) target cells. Less than 50% of these patients showed any increase in NK after treatment of their effectors with NDV or IFN and the level of cytotoxicity was only slightly above endogenous NK levels. The proportion of RA patients responding to augmentation by NDV or IFN did not differ significantly from the normal group (Fig. 3). While the cytotoxicity of NDV- and IFN-treated effectors on WI-L2 target cells did not differ from the normal group, the degree of augmentation to these stimuli was significantly lower than for the normal donors. This was largely due to the slightly elevated level of endogenous activity against this target cell exhibited by RA patient donors.

Sequential analysis of the NK activity of MS and SLE patients

Some MS, SLE and normal donors were retested for endogenous and augmented NK on repeated occasions at intervals of between 2 and 5 months. While there was some fluctuation in the level of endogenous NK on repeated testing, none of the donors showed any changes in their classification as being either NK+ of NK−. Of particular note, those patients who were NK− when tested initially continued to show defective NK with repeated testing over a period of several months. Similar consistency was seen in patients tested repeatedly for NDV- or IFN-augmented NK. The majority of donors who failed to respond to augmentation upon initial testing continued to be defective when retested.

The influence of disease activity and therapy on natural killing

Disease activity appeared to have a significant effect on the endogenous NK activity of MS patients (between group variance ratio = 2·92; $P < 0{\cdot}05$). Exacerbating, chronic progressive and stable MS patients exhibited significantly lower NK compared to patients in remission or to normal donors (Table 2). Similarly, there was a highly significant effect of disease activity (variance ratio = 15·08; $P < 0{\cdot}001$) and therapy (variance ratio = 13·49; $P < 0{\cdot}001$) on the endogenous NK of SLE patients, with the level of NK and the proportion of NK+ donors decreasing as disease activity and therapy increased (Table 2). Only one of five acute SLE patients tested showed any killing of K562 target cells and in this case the cytotoxic activity was only 4 LU. Patients receiving more than 20 mg of prednisone per day or a combination of prednisone and azathioprine showed a highly significant decrease in endogenous NK compared to normal donors. In contrast, patients receiving less than 20 mg per day of prednisone or no steroid therapy did not differ significantly in their endogenous NK from normal donors or from patients treated with higher steroid doses.The endogenous NK of RA patients did not vary significantly with disease activity (variance ratio = 2·36; $P < 0{\cdot}05$) (Table 2). However, therapy with steroids did significantly affect their endogenous NK (variance ratio = 7·35; $P < 0{\cdot}01$). While the level of NK was significantly lower in the group of RA patients receiving less than 20 mg of prednisone per day, there was no difference in the proportion of patients who were NK+.

Comparison of NDV- and IFN-augmented NK of both K562 and WI-L2 target cells between each of the disease activity and therapy subgroups showed similar results to those observed for endogenous NK (data not shown). As described above, MS patients undergoing acute exacerbation, chronic progression or stable disease showed significantly ($P < 0{\cdot}05$) less response to NDV and IFN than did patients in remission or than normal donors. The SLE patients showed a highly significant decrease ($P < 0{\cdot}001$) in both NDV- and IFN-augmented NK on both targets as disease activity and the level of steroid therapy increased. The augmented NK of RA patients did not vary significantly with disease activity, while there was a slightly significant ($P < 0{\cdot}05$) effect of therapy on the response to NK augmentation.

Comparison of IFN production with endogenous and augmented NK

Because the PBMC of many MS and SLE patients produce decreased amounts of IFN in response to various IFN inducers (Neighbour & Bloom, 1979; Neighbour *et al.*, 1981; Neighbour & Grayzel,

Table 2. The influence of disease activity and drug therapy on endogenous natural killing of K562 target cells

Group	No.	Cytotoxicity* ($\log_{10}$ LU)	LU	Proportion of NK+ donors† (%)
Normal	30	1·882±0·67	76	100
MS all	29	1·224±0·87‡	17	72‡
exacerbated	3	0·759±1·31§	6	33¶
remission	8	1·371±0·76	23	88‡
chronic progressive	9	1·278±0·87‡	19	78§
stable	9	1·194±0·93‡	16	67¶
Normal	28	1·850±0·65	71	100
SLE all	32	0·991±0·93	10	59¶
inactive	14	1·622±0·80	42	87‡
active	13	0·649±0·78¶	4	42¶
acute	5	0·111±0·25¶	1	20¶
no therapy	4	1·520±0·72	33	100
<20 mg prednisone	11	1·696±0·76	50	91
>20 mg prednisone	10	0·613±0·79¶	4	40¶
Prednisone & azathioprine	7	0·119±0·23¶	1	14¶
Normal	19	1·809±0·48	64	95
RA all	19	1·547±0·73	35	95
inactive	6	1·893±0·96	78	100
active	13	1·387±0·57	24	92
no therapy	8	2·057±0·72	114	100
<20 mg prednisone	11	1·175±0·49§	15	91

*Geometric mean±s.d.
†Donors whose NK activity did not differ significantly from the normal group mean ($P<0·05$).
‡Significantly different from normal group: $P<0·05$.
§Significantly different from normal group: $P<0·01$.
¶Significantly different from normal group: $P<0·001$.

1981), we compared endogenous and augmented NK of these patients with their ability to produce IFN. For each donor group, NDV-induced IFN data from the previous studies were compared by regression analysis with the level of endogenous and augmented NK assayed simultaneously. All correlations performed on data obtained for RA patients were not statistically different from the normal donor group and, for this reason, have been excluded from further discussion. There was no significant correlation between IFN-production and endogenous NK in the normal, MS or SLE donor groups. Indeed, some patients who failed to kill effectively were able to produce significant levels of IFN in response to stimulation, and conversely, several donors who failed to produce IFN did exhibit significant NK. While IFN production and both NDV- and IFN-augmented NK did not correlate among normal donors, there were significant correlations between these parameters in the MS and SLE patients (IFN vs NDV-NK: $r=0·483$, $P<0·01$; IFN vs IFN-NK: $r=0·557$, $P<0·05$). This result suggested that patients with a defective IFN response tended to be resistant to NK augmentation, but there were several noteworthy exceptions (see below).

Further analyses showed that endogenous NK did not correlate with either NDV- or IFN-augmented NK in the normal group although, NDV-augmented NK was highly correlated with IFN-augmented NK ($r=0·778$, $P<0·001$). The converse was seen in the patient groups. There was correlation between endogenous and IFN-augmented NK ($r=0·572$, $P<0·01$), but none between NDV- and IFN-augmented NK ($r=0·357$, $P>0·05$). Thus, many of the patients with

Table 3. Response profiles for IFN production, endogenous NK, NDV- and IFN-augmented NK of selected individuals

Donor	Group	IFN production	Endogenous NK	NDV-augmented NK	IFN-augmented NK
109	MS	+*	+	−†	−
112	MS	+	+	−	+
115	MS	+	−	+	+
118	MS	+	+	−	+
602	SLE	+	+	+	−
605	SLE	+	−	−	−
803	SLE	−	+	+	+
913	SLE	−	+	−	n.d.‡
916	SLE	+	−	+	+

*Normal, response is not significantly lower than normal group.
†Defective, response is significantly lower than normal group mean.
‡Not done.

defective endogenous NK also responded poorly to augmentation; and a failure of their NK to respond to NDV was not always accompanied by a non-responsiveness to IFN.

IFN-NK responses of individual patient donors
Because the various components of the IFN-NK system rarely correlated at the group level, data from selected individuals were analysed. Table 3 shows that some of these donors exhibited specific defects in either IFN production, endogenous NK or augmented NK, and that each function appeared, therefore, to be quite independent of the others. For example, donor No. 605 produced normal levels of IFN in response to NDV but failed to exhibit both endogenous and augmented NK. In contrast, donor No. 803 exhibited normal NK function but was unable to produce detectable IFN in response to NDV. The responses of donor No. 109 indicated that augmented NK is distinct from endogenous NK and that, for this patient at least, the ability to produce IFN is insufficient to augment NK effector function.

DISCUSSION

Previous reports of NK in patients with MS (Benczur *et al.*, 1980; Santoli *et al.*, 1981), SLE (Silverman & Cathcart, 1980; Hoffman, 1980; Oshimi *et al.*, 1980; Goto, Tanimoto & Horiuchi, 1980) and other chronic diseases with immunological involvement (Auer, Zeimer & Sommer, 1980; Koren, Amos & Buckley, 1978) used untreated effector cells and NK susceptible target cells (usually K562); an *in vitro* system in which the requirement for IFN-regulation appears to be minimal. In the present study, we confirm that endogenous NK is defective in many patients with MS and SLE and, in addition, report that IFN-dependent augmented NK is also abnormal in many donors suffering from these diseases.

Santoli *et al.* (1981) have recently reported that NK and IFN production was normal when tested in MS patients with stable or progressive disease; findings which differ from the data reported here and elsewhere (Benczur *et al.*, 1980). In view of our observation that the NK defect correlates with disease activity, differences in the clinical status of respective patient populations might yield conflicting results.

Repeated testing of both normal and patient donors showed that, while there were minor fluctuations in the levels of both endogenous and augmented NK, individuals maintained their status as having either normal or defective NK. When groups of individuals at various stages of disease activity were compared, it was observed that the more active the disease the lower the NK,

and that increasing steroid therapy was associated with decreased NK. This finding agrees with other investigators who have concluded that steroids are the probable cause of observed depression in NK seen in patients with SLE (Oshimi *et al.*, 1980). However, increasing steroid therapy parallels disease activity in the treatment of SLE and RA. Thus it is difficult to dissociate these two variables and selectively implicate one of them as being responsible for depressed NK. Some individual SLE patients exhibited deficient NK before treatment with steroids, while others receiving high doses showed normal as well as abnormal NK. In contrast, those RA patients receiving steroids exhibited significantly lower NK than untreated patients or normals and, although none of the MS patients were receiving any immunosuppressive therapy at the time of testing, some had defective NK. Therefore, while steroids appear to be associated with reduced NK at the group level, it is clearly not the sole cause of the defect in all individuals. A recent report of defective endogenous NK in untreated SLE patients supports this conclusion (Silverman & Cathcart, 1980).

There are several possible explanations for the observed defects in endogenous and augmented NK in MS and SLE donors. Many MS and SLE patients exhibit decreased responsiveness *in vitro* to inducers of both IFN-α and IFN-γ (Neighbour & Bloom, 1979; Neighbour *et al.*, 1981; Neighbour & Grayzel, 1981) and, because of the close functional interrelationship between IFN and NK, the inability to synthesize IFN in response to appropriate stimuli might, in turn, influence normal NK function. However, we have found that endogenous NK of K562 cells is largely independent of IFN synthesis during the *in vitro* cytotoxicity assay, and many patients whose effectors failed to lyse K562 cells did produce high titres of IFN in culture. In contrast, the inability to produce IFN in response to NDV could explain the lack of NK augmentation by this stimulus in some donors. It is apparent that many of the donors also have a selective defect in the sensitivity of their NK cells to IFN. We observed that while all MS patients responded to stimulation, the level of augmented NK after IFN treatment of effector cells remained lower than that seen in normal donors. In addition, half of the SLE patients tested failed to respond at all to exogenously applied high titres of IFN. The augmentation of NK by IFN is due in part to the recruitment of non-cytotoxic NK precursors (Minato *et al.*, 1980; Saksela, Timonen & Cantell, 1979; Silva, Bonavida & Targan, 1980), and to an increase in the rate of lysis and recycling of individual cytotoxic effector cells (Silva *et al.*, 1980; Ullberg & Jondal, 1981; Neighbour, unpublished observations). Therefore, patients who exhibit defective IFN-augmented NK may not only lack the NK cell itself, but the lytic activity of their functional NK cells may also be refractory to boosting by IFN. This particular observation is of importance when considering the possible therapeutic administration IFN to patients with chronic disease.

Additional explanations for the observed NK defects of these patients should also be considered. For example, circulating IFN in the serum of patients with acute SLE (Hooks *et al.*, 1979) might result in preactivation *in vivo* and subsequent 'exhaustion' of pre-NK cells. Because of circulating immune complexes or cytotoxic anti-lymphocyte antibodies, the NK effectors may be depleted from the circulation or be functionally impaired (Silverman & Cathcart, 1980; Hoffman, 1980; Goto *et al.*, 1980; Moretta *et al.*, 1979; Morimoto *et al.*, 1980; Santoli *et al.*, 1978; Reinherz *et al.*, 1980).

Inherited and acquired immunodeficiencies have yielded considerable information concerning some of the basic mechanisms of both cell-mediated and humoral immunity. Our experiments indicate that many patients with chronic disease such as MS and SLE are 'immunodeficient' in terms of their IFN-NK system. Analysis of IFN production and both endogenous and augmented NK by normal donors showed that these two functions can operate independently. This conclusion was further supported by data obtained from MS and SLE patients' cells, showing that the defects of IFN and NK function are often selective, with some individuals being deficient in only one component of the IFN-NK system. These data suggest that NK and IFN production either (a) are mediated by distinct subpopulations of cells; or (b) are independent functions of the same cell. Further experiments in this laboratory provide some additional evidence favouring the latter hypothesis.

Because of the possible role of IFN and NK in tumour surveillance and resistance to virus infections, it is tempting to speculate that defects in the IFN-NK system may predispose certain individuals to infection and, perhaps, to those chronic diseases of suspected persistent virus etiology

such as MS and SLE. However, in addition to defects in the IFN-NK system, many other immunological anomalies have been associated with these diseases. Because disease severity correlated with the extent of the NK defect, we contend that many of these anomalies result from a generalized disturbance of the immune system. Changes in lymphocyte traffic with possible sequestration of effector cells at sites of immunopathology, and the production of serum blocking factors or lymphotoxic antibodies are probable consequences of the disease. These in turn may contribute to and be manifest as altered immune responsiveness of lymphocytes in the peripheral circulation. However, while these immunological studies do not explain the underlying etiopathogenesis of chronic diseases, investigation of the immune responses of individuals with defined immunodeficiencies, as for example in the IFN-NK system, may add to our understanding of basic immunological mechanisms both *in vivo* and *in vitro*.

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