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Dysfunction of natural killer cells in multiple sclerosis: a possible pathogenetic factor

M. BENCZUR, G. Gy. PETRÁNYI, Gy. PÁLFFY,* M. VARGA, MARGARITA TÁLAS,† BOGÁTA KOTSY,* I. FÖLDES† & SUSAN R. HOLLÁN *National Institute of Haematology and Blood Transfusion, Budapest, * Department of Neurology and Psychiatry, Medical School, Pécs and † Microbiological Research Group of the Hungarian Academy of Sciences, Budapest, Hungary*

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SUMMARY

Natural killer (NK) cell activity, antibody-dependent cytotoxicity (ADCC) and the effect of interferon on NK activity was investigated in multiple sclerosis (MS) patients. NK activity measured against K-562 tumour cell line was found to be significantly low in MS patients, and this was most pronounced in the group of male patients with definitive disease. The response of NK cells to interferon proved to be impaired and almost no activation could be demonstrated in response to polyinosinic–polycytidylic acid (poly I:C). Preliminary data obtained by the determination of interferon production in several definitive MS cases reflect a defect in the interferon producing capacity of lymphocytes. The possible involvement of impaired NK cell function in the aetiopathogenesis of MS is discussed.

INTRODUCTION

A number of reports indicate that in the pathogenesis of multiple sclerosis (MS) virus infection(s) in association with other factors such as geographic and/or ethnic, genetic and immunologic (reviewed by Fog *et al.*, 1977) may be involved. Specific immune response to certain virus antigens could be demonstrated and non-specific immune parameters reflect an impaired immune function (reviewed by Batchelor, Compston & McDonald, 1978). Interest has been focused mainly on the high measles antibody titre of MS patients first reported by Ammitzboll & Clausen (1972). In recent years several reports have described the participation of natural killer (NK) lymphocytes in the non-specific defence against virus infection (McFarland, Burns & White, 1977; Trinchieri & Santoli, 1978; Welsh & Zinkernagel, 1977) besides their importance in the *in vivo* resistance against some tumours (Kiessling *et al.*, 1975; Petrányi *et al.*, 1974; Harmon *et al.*, 1977; Haller *et al.*, 1977; Herberman & Holden, 1978). It is suggested currently that interferon is the central regulator of NK activity in virus infections and some tumours (Santoli & Koprowski, 1979).

MS is associated with particular antigens of the HLA system; in Caucasians with HLA A3, B7 and DW2 (summarized by Fog *et al.*, 1977; Batchelor *et al.*, 1978). Similar association was reported in relation to NK cell function in healthy individuals; thus low NK activity correlated with HLA A3, B7, while high NK activity with HLA A2, B12 (Petrányi *et al.*, 1974; Varga *et al.*, 1975; Santoli *et al.*, 1976).

The present study shows that NK lymphocytes of MS patients display a low killer activity and an impaired response to interferon and interferon inducers. In view of this the possible pathogenetic role of NK dysfunction in MS is suggested.

Correspondence: M. Benczur, National Institute of Haematology and Blood Transfusion, 1113, Budapest, Daróczi ut 24, Hungary.

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MATERIAL AND METHODS

Disease definition. Eighty-eight patients with multiple sclerosis from the Department of Neurology and Psychiatry, Medical School, Pécs were studied. Of these fifty-three were females and thirty-five males, the average age being 34 and 38 years respectively. At the time of the examination twenty-two of the patients had exacerbation, thirty-seven were in remission* and the disease of twenty-nine patients was slowly progressive. During the 1-year study corticosteroids were given to only three patients and levamisol to eleven. All the other patients received vitamin B1, B12 and Baclofen (lioresal). Clinical diagnosis and the categories of the disease (definitive, probable, possible) were established according to Bauer's definition (Bauer, 1974).

NK and K cell activity assay. K-562 cell line (Lozzio & Lozzio, 1975) derived from a human myeloid tumour were used as target in the NK activity assay. Target cells were labelled with ^{51}Cr (Amersham, 100–150 μCi, 30 min, 37°C) and 1×10^4 cells, suspended in 50 μl, were transferred to U plates (Cooke, M-24 ART) for cytotoxic assay. Lymphocytes were collected from peripheral blood on Ficoll–Uromiro gradient, and monocytes were removed by carbonyl-iron treatment. For culture, the cell mixtures, prepared in 100 μl at 50:1, 25:1, 12:1, 6:1 and 3:1 lymphocyte to target cell ratios were brought to a 200 μl final volume using 50 μl medium (0·1% HSA–RPMI).

In the K cell activity assay the cultures were prepared from cell mixtures as above, but instead of the medium 50 μl 1:250 diluted anti-K562 rabbit serum was added to each well. Both reactions were read after a 4-hr incubation (at 37°C in an ASSAB CO_2 incubator) by determination of supernatant activity using the following formula for calculation of ^{51}Cr release:

$$\text{per cent specific release} = \frac{\text{c.p.m. in supernatant} - \text{c.p.m. in spontaneous release}}{\text{total incorporated activity}} \times 100.$$

Each lymphocyte-target cell combination was set up in four parallel cultures. Spontaneous release ranged between 6 and 12%. Based on the dose–response curve, cytotoxicity for a ratio of 20:1 was computed and used for comparison.

Treatment of lymphocytes with interferon and poly I:C. Human interferon prepared from leucocytes (3500 iu/ml) was kindly provided by Dr V. P. Kuznecov (Gamaleya Institute of Microbiology, Moscow).

Polyinosinic–polycytidylic acid (poly I:C) (Calbiochem) was used as interferon inducer (Field *et al.*, 1967).

Lymphocytes (1×10^7/ml) were pre-incubated either with interferon (in 1:20 dilution) for 30 min or with poly I:C (10 μg) for 18 hr at 37°C in 5% CO_2 atmosphere. The lymphocytes then were washed three times with 0·1% HSA RPMI medium by centrifuging at 900 *g* for 10 min and used in NK and K activity assays.

Supernates obtained after incubation of cells with poly I:C were investigated for the presence of interferon.

Interferon assay. The cytopathic effect–inhibition method was used for interferon determination (Finter, 1973).

Primary human embryo cell cultures growing in microtest TC plates (Falcon) were incubated with examined samples (0·05 ml) at 37°C in 5% CO_2 atmosphere for 18 hr. Vesicular stomatitis virus (Indiana strain) in a dose of 50–100 TCD_{50}/0·05 ml was used as challenge.

International standard interferon preparation was included in every test and interferon titres of samples investigated were expressed in international units (iu).

RESULTS

Peripheral blood lymphocytes of sixty-one MS patients without any grouping displayed a significantly low NK cell activity as compared to the age-matched controls (Table 1). Lowest activity was found in patients belonging to the definitive category of the disease according to Bauer (1974). ADCC activity of patients' lymphocytes against tumour targets gave similar results, though this kind of cytotoxicity was not as low as NK cell activity. Table 2 includes the same data according to sex distribution. In males the difference in NK activity between controls and patients is striking.

The activation of NK cell function by interferon showed a similar tendency. The response of NK cells to interferon treatment was significantly lower in MS patients than in healthy individuals (Table 1). The lowest activation was found in the patients with definitive disease, but activation was also low in the possible cases. The most pronounced difference to interferon stimulation could be demonstrated in male definitive MS patients in whom interferon induced only a moderate rise of *in vitro* NK cell activity as compared with the controls (Table 2).

Since it has been demonstrated that interferon inducers can increase efficiently NK cell function (Djeu *et al.*, 1979; Oehler *et al.*, 1978; Gidlund *et al.*, 1978), the lymphocytes of some MS patients and control subjects were incubated with poly I:C to detect subsequent NK cell function. As indicated in

* NK and K cell activity was measured within the first month of remission.

TABLE 1. NK cell activity and ADCC of MS patients and the effect of interferon

Group	No. of cases	NK cytotoxicity* (a)	NK cytotoxicity after interferon treatment (b)	Interferon augmentation (b–a)†	Antibody-dependent cytotoxicity*
Control	39	18·3±2·0‡	25·6±2·0	7·3±0·8	41·5±1·9
MS total	61	11·3±1·1§	15·3±1·3¶	4·0±0·6§	30·3±1·6¶
Definitive	43	9·4±1·1¶	12·7±1·1¶	3·7±0·7§	28·9±1·8¶
Probable	11	17·0±3·3	22·6±4·4	3·6±1·5	32·3±3·8**
Possible	7	14·3±4·0	18·0±5·6	3·8±1·8	37·5±5·1

* Cytotoxicity expressed in per cent specific release at 20:1 lymphocyte–target cell ratios.
† Individually calculated.
‡ ±s.e.
§ $0{\cdot}01 > P > 0{\cdot}001$ compared to control value.
¶ $P < 0{\cdot}001$ compared to control value.
** $0{\cdot}05 > P > 0{\cdot}01$ compared to control value.

TABLE 2. NK cell activity and ADCC of MS patients and controls related to sex difference

Group	Sex	No. of cases	NK cytotoxicity*	Interferon augmentation of cytotoxicity*	Antibody-dependent cytotoxicity*
Control	Males	13	23·8±3·5†	8·8±1·8	44·5±4·0
	Females	26	15·5±1·6	6·8±0·9	35·8±2·3
MS	Males	15	8·6±2·1‡	3·4±0·7§	28·1±3·8§
	Females	25	10·5±1·3¶	4·0±0·9¶	29·4±2·0¶

* Cytotoxicity expressed in per cent specific release at 20:1 lymphocyte–target cell ratios.
† ±s.e.
‡ $P < 0{\cdot}001$.
§ $0{\cdot}01 > P > 0{\cdot}001$ compared to matched control.
¶ $0{\cdot}05 > P > 0{\cdot}01$.

Table 3, in contrast to healthy individuals poly I:C did not enhance NK cell activity of MS patients. (It should be noted that lymphocytes of each group were incubated at 4°C for the same period—18 hr—in albumin-supplemented medium without FCS. This long incubation period markedly depressed the NK cell activity, probably as a consequence of cell death or functional impairment of certain lymphocyte subpopulations. The effect of poly I:C, therefore, could not be attributed only to its interferon-inducing capacity but also to some kind of protective effect.)

To elucidate whether poly I:C under the given experimental conditions induces production of interferon, lymphocytes of controls and of patients with a clinically definitive form of MS were incubated with poly I:C at 37°C for 18 hr and the supernates subsequently were tested for the presence of interferon. As is evident from Table 4, low interferon titres were observed in all samples investigated. Apparently, lymphocytes of MS patients produce less interferon than those of controls.

TABLE 3. NK cell activity of MS patients and controls after poly I:C treatment

Groups	No. cases cases	NK cytotoxicity* without poly I:C treatment†	NK cytotoxicity* after poly I:C treatment‡
Control	5	5·0±0·8	10·1±2·0
MS	9	4·2±0·9	3·9±0·9§

* Cytotoxicity expressed in per cent specific release at 20:1 lymphocyte–target cell ratios.

† NK activity of lymphocytes incubated for 18 hr in serum-free medium at 4°C.

‡ NK activity of lymphocytes incubated for 18 hr in 100 g/ml poly I:C containing serum-free medium.

§ $0{\cdot}01 > P > 0{\cdot}001$.

All values± s.e.

TABLE 4. Induction of interferon by poly I:C in lymphocytes of MS patients and controls

Groups	No. of subjects	Titre of interferon* (iu) (0·05 ml)	Mean titre of interferon
Control	10	40,10,40,160,40 80,10,40,40,40	50±14
MS†	7	10,40,10,10, 10,40,10	13±6‡

* Cells (1×10^7) were incubated with 10 μg of poly 1:C at 37°C for 18 hr. After centrifugation the supernates were tested for the presence of interferon.

† Lymphocytes of patients with clinically definitive form of multiple sclerosis.

‡ $0{\cdot}05 > P > 0{\cdot}02$ in comparison to the control.

DISCUSSION

Our knowledge of the aetiology and pathogenesis of MS is very limited, the observed genetic and epidemiologic–geographic factors, however, seem to be important. Previously, infectious agents were considered as possible factors in the pathogenesis of MS. More recent observations by Mitchell *et al.* (1978), Prasad *et al.* (1977) and Ebina *et al.* (1979) favour the possible pathological role of viruses or virus-like substances. According to other reports, however, there is no evidence for a multiple sclerosis-associated agent (Gravell *et al.*, 1978).

In the light of current series of experiments the importance of NK lymphocytes in the defence against virus-infected cells becomes the focus of interest (McFarland *et al.*, 1977; Welsh & Zinkernagel, 1977; Gidlund *et al.*, 1978). Trinchieri *et al.* (1978) demonstrated that virus-transformed cells induce an increase of human NK cell activity; the same was reported to occur as a result of interferon treatment (Herberman, Ortaldo & Bonnard, 1979). According to preliminary observations, there is an increase in NK activity after influenza vaccination (Santoli & Koprowski, 1979).

Interferon inducers such as poly I:C, Tilorone, *Corynebacterium parvum* or BCG cause a similar

increase of NK cell activity (Djeu *et al.*, 1979; Oehler *et al.*, 1978; Gidlund *et al.*, 1978; Since anti-interferon treatment can block the effect of poly I:C it seems to be well documented that in these cases NK activation is mediated by interferon (Gidlund *et al.*, 1978). We demonstrated that NK cell activity is impaired in MS patients. It seems to be of interest that the most pronounced difference in cytotoxicity was found between the group of male patients and their matched controls. A similar study is mentioned in the most recent paper of Santoli & Koprowski (1979). In opposition to our findings, they emphasize normal or even elevated NK activity in MS patients. The question remains open whether NK activity is also changed in non-MS neurological disorders? This problem needs further investigation.

If one takes into consideration the fact that interferon may play an important role in the modification of NK cell activity (Herberman *et al.*, 1979; Santoli & Koprowski, 1979) the impairment of NK cell function in MS could be ascribed to (i) primary genetic dysfunction of NK cells; (ii) a secondary suppression by the virus; (iii) defective interferon production and its failure in modulating NK function.

The evidence concerning the HLA-association of the NK function favours the first explanation; moreover, our results obtained by interferon treatment underline the role of interferon-modulation.

The finding that male patients whose NK cell population was hardly able to respond to interferon treatment, deserves particular attention.

Preliminary data obtained in connection with our eight MS patients seem also to indicate the possible defect in interferon production. All these may reflect NK cell dysfunction, as NK activity and interferon production might be the functional property of the same cell population (Santoli & Koprowski, 1979). Basic questions, such as virus aetiology of MS, significance of NK cell function in the *in vivo* resistance against virus infection in humans, association of NK dysfunction with HLA and impaired humoral and cell-mediated immune parameters, and the role of sex differences in the clinical course of the disease could not be answered to date. Our analysis concerning HLA revealed that low NK cell function of MS patients was associated with HLA DRW 2 antigen, rather than with HLA B 7 (manuscript in preparation).

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