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ABNORMAL MACROPHAGES AND NK CELL CYTOTOXICITY IN HUMAN SYSTEMIC LUPUS ERYTHEMATOSUS AND THE ROLE OF INTERFERON AND SERUM FACTORS

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Summary. — Macrophage (MO) and natural killer (NK) cell mediated cytotoxicity to K_{562} target cells were strikingly decreased in patients with systemic lupus erythematosus (SLE). SLE NK cells failed to release soluble factor(s) for lysing the targets. IFN-induced enhancement of both types of cytotoxicity was impaired. NK cells from healthy subjects kept their activity in culture with or without IFN for more than six days whereas SLE NK cell activity declined to zero at day 3. So, the increased IFN level of many SLE patients and a possible prior IFN priming effect seemed unrelated to the insensitivity to exogenous IFN *in vitro*. Inhibition factor(s) of SLE serum supressed NK cytotoxicity in the presence of IFN whereas IFN sensitivity of MO remained unaffected indicating the complex regulation by serum components of immune reactions.

Key words: *Systemic lupus erythematosus*; *SLE-sera; macrophage and natural killer cell mediated cytotoxicity*; K_{562} *cells*; *human interferon* (*IFN*)

Introduction

Spontaneous cytotoxicity was originally thought to be an artifact of *in vitro* cytotoxicity assays. Its importance as a host defence mechanism is becoming increasingly evident. Cytotoxic mechanisms against both tumour cells and microorganisms mediated by MO and NK cells have been shown to be enhanced by interferons (Schultz and Chirigos, 1978; Herberman *et al.*, 1979, 1982).

NK cell function is abnormal in a number of human diseases including SLE and cancer (Kadish *et al.*, 1981; Merrill *et al.*, 1982; Goto *et al.*, 1982). This is complicated by the finding that many patients with SLE have elevated levels of circulating IFN (Preble *et al.*, 1982; Panem *et al.*, 1983). It is known that IFN-induced enhancement of NK activity in SLE is impaired (Fitzharris *et al.*, 1982; Sibbitt *et al.*, 1983). The experiments of Ohara *et al.*

(1983) have shown that a given lower antibody-dependent cell mediated cytotoxicity (ADCC) of lymphocytes in SLE-patients is compensated by higher ADCC of their polymorphonuclear cells (PMN).

This investigation was undertaken to elucidate a possible similar role of MO in the impaired NK activity of SLE-patients. No compensating effect could be shown. The addition of IFN and autologous serum of SLE-patients results in a synergistic decrease of cytotoxicity. SLE NK cells represent a functional unability to release cytotoxin to target cells resulting in a diminished cytotoxic activity.

Materials and Methods

Twenty patients suffering from active SLE were investigated and all fulfilled four or more criteria for diagnosis of the disease (Cohen *et al.*, 1971). Healthy humans served as controls.

Effector cells. Human peripheral blood cells (PBL) were isolated from heparinized whole blood (20 ml per patients) by Ficoll-Hypaque density gradient centrifugation according to Boyum (1976) and the resulting cell population was divided into equal parts. In one half, glass adherent cells were removed by incubating the PBL in a tissue culture bottle at 37 °C for 40 min and washing twice with warm phosphate buffered saline (PBS). The titre of adherent and nonadherent PBL was calculated by means of blood chamber counting before and after incubation, and adjusted to 2×10^6 adherent or nonadherent cells per ml in RPMI 1640 medium supplemented with 10 % foetal calf serum (FCS) and 100 IU penicillin/ml and 100 µg streptomycin/ml.

All natural cytotoxic effects of the nonadherent cell population could be neutralized by anti-asialo GM_1 antibodies (prepared by Dr. Wollweber, CIMET, Jena) indicating pure NK cell activity in this preparation.

Tumour cells. 3×10^6 K_{562} tumour cells (human erythroid myeloid cell line) were labelled with 10 µl Na_2 $^{51}CrO_4$ — 3.7 MBq (Central Institute for Nuclear Research Dresden-Rossendorf, G.D.R.) in 200 µl basal Eagle medium (BEM) for 1 hr. The target cells were washed three times with BEM (37 °C) and adjusted to 2×10^5 cells per ml.

Interferon. Human leukocyte IFN (Huleu-IFN) was provided by Dr. Fuchsberger (Institute of Virology, Slovak Academy of Sciences, Bratislava) and diluted in RPMI 1640 medium (500 IU/50 µl).

^{51}Cr release assay. 0.1 ml effector cell suspension containing 2×10^5 adherent (MO activity) or nonadherent PBL (NK activity) were cocultured with 1×10^4 ^{51}Cr labelled K_{562} targets with or without 500 IU IFN per well 16 hr at 37 °C under 5 % CO_2 atmosphere in microculture plates (Flow Laboratories, F.R.G.). Since K_{562} cells are not sensitive to IFN action the direct effect of IFN on these cells could be ruled out as a cause of tumour cell susceptibility to the cytotoxic effects of IFN-activated MO or NK cells.

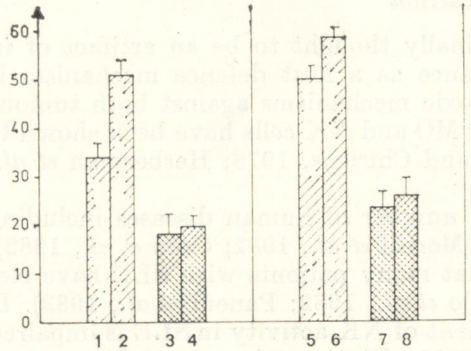

Fig. 1.
MO and NK-cell-mediated cytotoxicity of PBL from healthy persons (filled columns 3, 4, 7, 8) and SLE patients (hatched columns 1, 2, 5, 6) with or without 500 IU IFN/ 2×10^5 effector cells after 16 hr ^{51}Cr release assay
Columns 1 + 3: MOs; columns 5 + 7: NK cells only; columns 2 + 4: MOs + IFN; columns 6 + 8: NK cells with IFN. Mean values from 20 SLE patients and 10 healthy controls and standard error are shown.
Both MO and NK-cell-mediated cytotoxicity are diminished in SLE, and responsiveness to IFN is impaired.

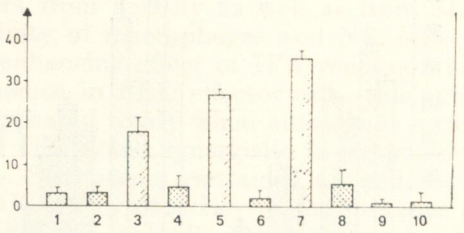

Fig. 2.
Lytic capacity of cell-free supernatants from 16 hr cultured K_{562} and/or NK cells cultured for 16 hr with and or without 500 IU IFN/2×10^5 effector cells against ^{51}Cr labelled K_{562} tumour cell targets after 16 hr cocultivation Columns 1 + 2: supernatants from NK cells only; columns 3 + 4: NK cells cocultivated with K_{562} cells; columns 5 + 6: NK cells cocultivated with IFN; columns 7 + 8: NK cells cocultivated with IFN + K_{562}; column 9: K_{562} cells only; column 10: K_{562} cells cocultivated with IFN.
Mean values from 6 controls and 6 SLE patients and standard error are shown. Both IFN and tumour cells only induced release of NK cell-derived soluble cytotoxic factor(s) into the medium by cocultivation with healthy NK cells (filled columns 2, 4, 6, 8, 10). These acted synergistic ally. The ability of SLE NK cells to release such factor(s) was drastically impaired (hatched columns 1 3, 5, 7, 9).

In some experiments 25 µl heat-inactivated autologous serum was added per well resulting in a final serum concentration together with the medium containing FCS of 20 per cent. Cells were removed by a supernatant harvesting system (Flow Laboratories, F.R.G.). The radioactivity of the supernatants was estimated by means of a gamma-counter (Packard Prias, PGD). All samples were run in sextuplet.
The percentage of NK or MO activity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{\text{Exp. } ^{51}Cr \text{ release} - \text{spont. } ^{51}Cr \text{ release}}{\text{Max. } ^{51}Cr \text{ release} - \text{spont. } ^{51}Cr \text{ release}} \times 100$$

Mean values and standard errors were plotted out.

Preparation of the NK cell-derived cytotoxic factor. Nonadherent PBL from SLE patients or controls were cocultured with or without cold K_{562} cells with or without IFN under the same conditions as mentioned in the ^{51}Cr release assay but using double cell and IFN concentrations. Supernatants were prepared by centrifugation at 110 × g, +4 °C and their tumour cell lytic capacity determined by 1 : 1 cocultivation with ^{51}Cr labelled K_{562} cells (1×10^5/0.2 ml) 16 hr and subsequent estimation as mentioned above.

Long term cultivation. Nonadherent PBL (2×10^6/ml) from SLE patients and controls were cultivated six days in RPMI 1640 medium supplemented with 10 % FCS and antibodies with or without 500 IU IFN/ml in plastic Petri dishes (KOH-I-NOOR Hardtmuth, O.P., České Budějovice) under CO_2-atmosphere. The medium was changed once a day. The cells were used for ^{51}Cr release assay by 16 hr, 24 hr, 3 and 6 days in culture.

Results

Healthy control persons and SLE patients showed significant differences in both MO and NK cell mediated cytotoxicity to K_{562} tumour target cells. The lytic capacity was strikingly reduced and the responsibility to IFN as a cytotoxicity elevating factor was impaired (Fig. 1). This defect in IFN response persisted despite its increasing concentrations.

NK cells became cytotoxic for target cells by releasing a factor(s) after the contact with tumour cells or IFN or both, which acted in a synergistic manner. This effect was disturbed in SLE patients (Fig. 2). The NK cell derived lytic factor(s) from healthy persons could be stored at +4 °C for

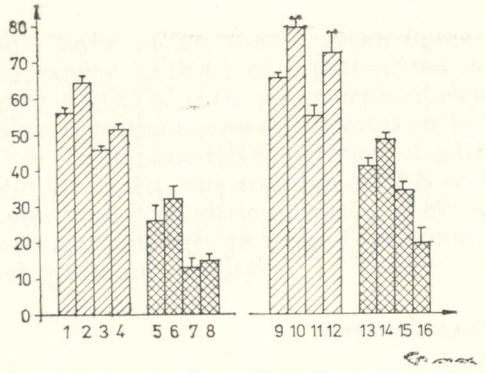

Fig. 3.
Cytotoxicity of NK cells from healthy and SLE donors to K_{562} targets after cultivation for 16 hr, 24 hr, 3 days and 6 days with 500 IU IFN/2×10^5 effector cells (full lines) and in the absence of IFN for for a 16 hr cytotoxicity assay (interrupted lines)
Squares represent healthy NK cells and circles SLE NK cells. Mean values from 3 SLE patients and 3 healthy persons as control and standard error are shown. NK activity and IFN responsiveness of healthy control patients remained almost constant for three days and show reduced levels at day six whereas SLE NK activity started to decline already after 16 hr. No functional maturation appeared in SLE NK cells.
Abscissa: time in culture; ordinate: cytotoxicity (%).

three days showing declined activity at day 6; it was sensitive to the freezing/thawing procedure. The functionally immature state of SLE NK cells could not be restored by long time cocultivation with IFN as known for healthy NK-precursors. The cytolytic capacity was even diminished after three days (Fig. 3). In contrast, NK cells from healthy control patients kept their activity nearly constant up to three days; then it declined to 50 per cent of the starting activity (day 6). The sensitivity to IFN was not abolished whereas no cytotoxicity enhancing effect was observed for SLE NK cells.

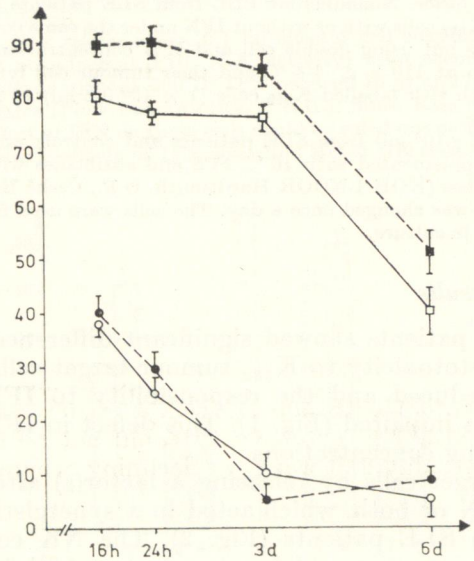

Fig. 4.
Cytotoxicity of healthy and SLE NK cells and MOs alone, cocultivated for 16 hr with and without 500 IU IFN/2×10^5 effector cells and/or 10 % autologous serum
Columns 1—4: healthy MOs alone, MOs with IFN, MOs with serum, and MOs with serum and IFN, columns 5—8: SLE MOs, columns 9—12: healthy NK cells, and columns 13—16: SLE NK cells in the same manner.
Autologous sera suppress the cytotoxic activity of MOs and NK cells. Additionally, serum factor(s) from SLE patients acts synergistic with IFN in suppressing NK but not MO mediated cytotoxicity.

As shown in Fig. 4, autologous sera from healthy as well as from SLE patients suppressed the cytotoxic activity of macrophages and NK cells in the *in vitro* assay. The cytotoxicity enhancing effect of IFN remained unchanged at healthy effector cells. Whereas in SLE effector cells, this cytotoxicity enhancing effect of IFN disappeared totaly when autologous serum was used. In opposite, SLE sera and IFN acted synergistic in suppressing NK cell mediated cytotoxicity. Thus, SLE serum contained an additional factor which significantly inhibited NK cell cytotoxicity in the presence of IFN as compared in the absence of IFN. No such effect was observed with NK cells from healthy controls.

Discussion

The attention of many clinical investigations has become directed to cellular processes in autoimmune diseases such as SLE etc. Many patients suffering from SLE showed decreased NK activity in spite of high levels of circulating IFNs (Preble *et al.*, 1982; Goto *et al.*, 1982; Panem *et al.*, 1983). Higher ADCC values of PMN compensating the lower ADCC of lymphocytes were found by Ohara *et al.* (1983). This study was undertaken in order to investigate a possibly similar compensating effect of cytotoxic MO to decreased NK activity. But, both MO and NK cell-mediated cytotoxicity to K_{562} cells may be strikingly decreased in SLE patients (Fig. 1). In comparison with healthy persons, the mean MO and NK cytotoxicities are reduced by 15—20 and 25—30 per cent, respectively. Whereas in healthy probands MO as well as NK activity have been increased by 500 IU IFN/2 $\times$ 10^5 effector cells *in vitro*. SLE MO and NK cells remained without significant effect. Which are the reasons for such decreased cytotoxic activities? It is known that NK cell-mediated lysis is caused by soluble factor(s) released after recognition of target cells (Wright *et al.*, 1983). We investigated whether this mechanism was disturbed in NK cells from SLE patients. Supernatants from NK cells alone, cocultivated with and without tumour cells and/or IFN, and from tumour cells alone as controls were prepared and tested for their lytic capacity to ^{51}Cr labelled K_{562} cells. As shown in Fig. 2, both tumour cells and IFN were able to induce the release of cytotoxic NK products in healthy probands but not in SLE NK cells. There was even a synergistic effect between both stimulants. The lack in the NK cells activity of SLE patients might be caused by failure or by immature state of large granular lymphocytes as proposed by Egan *et al.* (1983) and Mandeville *et al.* (1983). It is known that the number of mature NK cells can be increased by IFN influencing pre-NK cells (Flexman and Shellam, 1982). We tried to mature the SLE NK cells by *in vitro* cultivation with 500 IU IFN/2 $\times$ 10^5 cells. The results in Fig. 3 indicate that SLE NK cytotoxicity could not be increased by cocultivation with IFN until day 6, it was even declining, whereas NK cells activity of healthy persons remained unchanged until cultivation and was enhanced on day 3 by addition of IFN during the 16 hr cytotoxicity assay.

It can be concluded that the intensitivity to IFN of SLE NK cells cannot be related to previous IFN primaring resulting from elevated levels of endogenously circulating IFN as proposed by Sibitt *et al.* (1983). That the IFN action is limited only to special NK subset populations was shown by Fitzgerald *et al.* (1983). In contrast, the diminished cytotoxicity of MOs in SLE patients and their inability to become activated by IFN can be explained by the presence of endogenous IFN. Becker (1984) could provide evidence that IFN prevents the development of monocytes to MOs.

For NK cytotoxicity, additional factors must be included. For this reason we investigated the influence of heat inactivated autologous sera in the NK cell mediated cytotoxicity. As shown Fig. 4 the contents of 10 % autologous serum in the cultural medium from healthy macrophages or NK cells as well as SLE macrophages or NK cells caused a suppression of the cytotoxic outcome in the *in vitro* assay. But, whereas in healthy persons the ability of NK cells to become activated by IFN was not influenced by autologous sera, SLE NK cells cocultivated with SLE serum and IFN resulted in additional suppression of NK cytotoxicity (15 % more than without IFN). In MO cytotoxicity test autologous SLE serum had no shown additional effects in the presence of IFN. It can be summarized:

1. Both macrophage and NK cell mediated cytotoxicity were diminished in patients suffering from SLE. It means, the low level of NK cytotoxicity is not compensated by a higher level of macrophage cytotoxicity. This impaired cytotoxicity of MOs seems to be caused by the presence of endogenous IFN (Becker, 1984).
2. SLE NK cells were not able to release cytotoxins to target cells. A possible immature state of SLE large granular lymphocytes could not be improved by long term cultivation with or without IFN, indicating a principial failure of SLE NK cells.
3. Autologous sera suppressed the cytotoxic outcome of MOs and NK cells.
4. SLE NK activity was, in addition, suppressed by a serum factor(s) acting in cooperation with the circulating IFN.

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