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Functions of Human Neutrophilic Granulocytes After In Vivo Exposure to Interferon Alpha

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The ability of neutrophilic granulocytes to phagocytize yeast particles and to reduce Nitro Blue Tetrazolium at rest and on activation with bacterial stimuli was monitored in 32 patients receiving treatment with human interferon alpha. The ability of these cells to attach to and ingest yeast particles was not altered to any major extent during 1 year of interferon treatment. In most patients, the Nitro Blue Tetrazolium-reducing activity increased after the first injection of interferon. During prolonged treatment with interferon alpha, 1 week to 1 year, granulocytes activated with bacteria exhibited a reduced Nitro Blue Tetrazolium activity in most patients.

Interferon (IFN) can be detected in the serum and other body fluids of patients with a variety of diseases, such as viral infections (9, 18, 24), bacterial infections (20), autoimmune disorders (12), and malignant tumors (10). The role of this endogenously produced IFN is only partly understood, since IFN has been shown to affect cellular functions in numerous ways (1). During recent years IFN has been administered to patients with various diseases (23). These studies have given us an opportunity to monitor the in vivo effects of IFN on immunological functions. Some of these functions have been found to be affected by IFN administration (6–8), whereas others were not affected to any measurable extent (4, 5, 7).

Neutrophilic granulocytes participate in the defense of the host against viral and bacterial infections. These cells have also been suggested to participate in the defense of the body against tumors (16). The facts that infectious diseases and tumors induce the production of endogenous IFN and that IFN is given to patients with viral infections and tumor diseases led us to study the influence of IFN-α therapy on some functions of granulocytes, namely, phagocytosis and reduction of Nitro Blue Tetrazolium (NBT). The latter function reflects the oxidative metabolism of cells, which is known to be enhanced during phagocytosis (21). This function was measured in the absence or presence of bacterial stimuli and autologous plasma.

MATERIALS AND METHODS

Patients and controls. A total of 32 patients were included in the study, 9 with hypernephroma, 7 with ovarian carcinoma, 5 with prostatic carcinoma, 2 with condylomata accuminata, 2 with osteosarcoma, 2 with multiple warts, and 1 each with Waldenström's macroglobulinemia, carcinoma of the base of the tongue, neuroblastoma, giant cell sarcoma, and papillomas of the trachea. There were 13 males and 19 females. The mean age was 52 years.

The granulocyte functions of 10 healthy donors were tested for comparison. These donors, 5 males and 5 females, had a mean age of 32 years.

IFN preparations. The IFN-α preparations were derived from human peripheral blood leukocytes exposed to Sendai virus and partially purified to specific activities of ca. 10^6 U of IFN per mg of protein as previously described (3). The antiviral activities of the preparations were determined by assaying inhibition of plaques induced by vesicular stomatitis virus in U-cells (22) and were expressed in international units by comparison with the international reference preparation 69/19. The IFN preparations were obtained from the Finnish Red Cross Blood Transfusion Service or from the Central Public Health Laboratories, Helsinki, Finland. All patients were treated by daily intramuscular injections of 3×10^6 U of IFN.

Preparation of granulocytes. Blood samples were drawn before initiation of IFN therapy and after 1 day, 1 week, 3 months, 6 months, 9 months, and 12 months of treatment.

Granulocytes were separated and tested immediately after the blood had been drawn. The separation from venous blood by centrifugation on a layer of Ficoll-Isopaque was performed according to the method of Böyum (2). Samples of crystal violet-stained cells were counted in a Burker chamber. The remaining cells were suspended in Eagle minimal essential medium to a density of 12×10^6 cells per ml.

Attachment and ingestion of fluorescein-labeled yeast particles. A modification of the method described by Hed (11) was used. On each of three glass slides 0.1 ml of the granulocyte suspension was placed, and the cells were allowed to adhere for 30 min at 37°C. The slides were then rinsed with Ringerdex solution at 37°C. Thereafter, 0.1 ml of Eagle minimal essential medium containing 4×10^7 particles of yeast (*Saccharomyces cerevisiae*) per ml, which was labeled with fluorescein isothiocyanate and opsonized in fresh human serum, was added to each slide. The preparations were then incubated for 15 min at 37°C. The reaction was terminated by placing the slides in Ringerdex solution at 4°C, followed by staining with crystal violet. Individual cells were then examined for the number of ingested particles, distinguishable by their fluorescence, and for the number of attached particles, colored dark by crystal violet, with a fluorescence microscope. The results were expressed as the number of attached or ingested particles per granulocyte. At least 30 cells were scored on each slide. The mean value for each slide was calculated, and the grand mean of the triplicates was determined.

NBT test. This method has been described elsewhere (14). Briefly, 0.4 ml of the granulocyte suspension and 0.3 ml of Eagle minimal essential medium or autologous plasma were

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TABLE 1. Comparison between 10 healthy donors and 32 patients (before IFN treatment) in regard to the phagocytic and NBT-reducing activity of their neutrophilic granulocytes[a]

Test	Bacterial stimuli	Autologous plasma	Values for patients	Values for healthy donors
Ingestion			2.8 ± 0.1	2.6 ± 0.1
Attachment			1.7 ± 0.1	1.9 ± 0.1
NBT	−	−	0.18 ± 0.01	0.35 ± 0.06
NBT	−	+	0.23 ± 0.02	0.34 ± 0.12
NBT	+	−	0.70 ± 0.04	0.84 ± 0.10
NBT	+	+	1.44 ± 0.06	1.31 ± 0.11

[a] The tests for NBT reduction were performed in the absence (−) or presence (+) of bacterial stimuli and autologous plasma, and the values are expressed as optical densities. The values for ingestion and attachment are expressed as particles per cell. The mean ± standard error is presented.

transferred to a plastic tube, and 0.5 ml of an NBT solution (0.1% of NBT in Hanks balanced salt solution) was added. For measurement of NBT reduction upon activation with bacteria, 1.5×10^9 heat-killed *Escherichia coli* were added to some of the tubes. After incubation for 30 min under continuous agitation in a 37°C water bath, the reaction was interrupted by addition of 2 ml of 0.5% HCl. The formazan produced was extracted with dimethylsulfoxide, and the optical density of the extract, expressed in arbitrary units, was determined.

Statistical analyses. Statistical analyses were performed with Student's *t* test.

RESULTS

Granulocyte functions in patients and healthy donors. When the phagocytic and NBT-reducing activities of granulocytes from 32 patients, before treatment, were compared with those of granulocytes from 10 healthy donors, the mean phagocytic activity of the patient and the healthy donor granulocytes was found to be similar (Table 1). As for reduction of NBT in the absence of bacterial stimuli and autologous plasma, the capacity of the patient granulocytes was lower as compared with that of healthy donor granulocytes ($P < 0.01$, according to Student's *t* test for independent samples; Table 1). On stimulation of granulocytes with bacteria, no major difference in NBT reduction between patients and healthy donors could be observed (Table 1).

Attachment and ingestion of yeast particles. During a treatment period of 12 months, no major change in the ability of peripheral granulocytes to ingest yeast particles was observed (Table 2). The mean number of yeast particles adhering to granulocytes was also found to be essentially unaltered during long-term IFN therapy (Table 2). The same results were obtained when the patient group was divided according to disease (data not shown).

TABLE 2. Change in the ability of neutrophilic granulocytes to ingest and attach to yeast particles during IFN therapy[a]

Time after start of IFN therapy	No. of patients	Ingestion (particles per cell)	Attachment (particles per cell)
1 day	31	−0.10 ± 0.09	+0.17 ± 0.15
1 wk	27	±0.00 ± 0.10	+0.06 ± 0.13
3 mo	22	−0.19 ± 0.19	−0.18 ± 0.17
6 mo	13	+0.04 ± 0.15	−0.14 ± 0.22
9 mo	7	+0.09 ± 0.20	−0.16 ± 0.29
12 mo	4	−0.40 ± 0.17	−0.48 ± 0.42

[a] Values obtained during treatment are expressed in relation to pretreatment values (see Table 1), i.e., the value during treatment minus the value before treatment. The mean ± standard error is presented. None of the changes was statistically significant.

TABLE 3. Change in the capacity of resting neutrophilic granulocytes to reduce NBT during IFN therapy[a]

Time after start of IFN therapy	No. of patients	NBT values with no plasma	NBT values with plasma
1 day	31	+0.14[b] ± 0.03	+0.21[b] ± 0.04
1 wk	27	+0.07[c] ± 0.03	+0.07[d] ± 0.03
3 mo	21	± 0.00 ± 0.03	+0.05 ± 0.03
6 mo	13	+0.02 ± 0.04	+0.10 ± 0.06
9 mo	8	−0.02 ± 0.04	+0.08 ± 0.04
12 mo	4	−0.10 ± 0.08	−0.14 ± 0.07

[a] Tests were performed in the absence or presence of autologous plasma. Values obtained during treatment are expressed in relation to pretreatment values (see Table 1), i.e., the value during treatment minus the value before treatment. The mean ± standard error is presented.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.

Reduction of NBT in the absence of bacterial stimuli. With few exceptions, the ability of granulocytes to reduce NBT at rest was increased after the first injection of IFN-α (Table 3). The change was statistically significant ($P < 0.001$). Also, after 1 week of IFN therapy the NBT-reducing capacity of granulocytes at rest was significantly increased (Table 3). The change was statistically significant ($P < 0.01$ and 0.05 in the absence and presence, respectively, of autologous plasma). After 3, 6, 9, and 12 months of treatment, there were no significant changes in the NBT-reducing capacity of resting granulocytes (Table 3).

Reduction of NBT in the presence of bacterial stimuli. One day after initiation of IFN therapy, the capacity of neutrophilic granulocytes to reduce NBT in the presence of bacterial stimuli was augmented in a majority of the patients (Table 4). During prolonged treatment, 1 week to 1 year, this granulocyte function was reduced in most patients studied (Table 4). The decrease was statistically significant after 3 months of treatment ($P < 0.001$ and 0.01 in the absence and presence, respectively, of autologous plasma).

DISCUSSION

This study shows that the NBT-reducing activity of neutrophilic granulocytes is affected by IFN in vivo. One day after initiation of treatment, this activity was increased in most patients (Tables 3 and 4). During prolonged treatment, however, NBT reduction in the presence of bacterial stimuli was found to be decreased in most patients (Table 4). This was not due to a decline in the functional activity of the patient granulocytes caused by progression of the disease, since this function of granulocytes decreased also in patients in whom the tumors remained clinically stable or regressed during IFN treatment. Bacterial infections of the respiratory tract are often preceded by viral infections (19), and it has even been suggested that viruses are obligatory for bacterial infections to occur (17). The mechanism for the predisposing effects of viruses in bacterial superinfections is not fully understood, but may be explained by effects on ciliated cells,

TABLE 4. Change in the capacity of neutrophilic granulocytes to reduce NBT in the presence of bacterial stimuli during IFN therapy[a]

Time after start of IFN therapy	No. of patients	NBT values with no plasma	NBT values with plasma
1 day	32	+0.09[b] ± 0.04	+0.11 ± 0.07
1 wk	27	−0.09 ± 0.05	−0.11 ± 0.10
3 mo	21	−0.27[c] ± 0.05	−0.32[d] ± 0.11
6 mo	12	−0.09 ± 0.12	−0.35 ± 0.19
9 mo	8	+0.08 ± 0.06	−0.24 ± 0.15
12 mo	4	−0.26 ± 0.15	−0.40 ± 0.31

[a] Tests were performed in the absence or presence of autologous plasma. Values obtained during treatment are expressed in relation to pretreatment values (see Table 1), i.e., the value during treatment minus the value before treatment. The mean ± standard error is presented.

[b] $P < 0.05$.

[c] $P < 0.001$.

[d] $P < 0.01$.

on mucus-producing cells, or on cells of the immune system. It has previously been reported that the NBT-reducing activity of granulocytes is enhanced early at the acute stage of influenza A infection (14). Based on our present findings, one may hypothesize that this enhancement of NBT reduction is due to the endogenous production of IFN. Subsequently, the continuous exposure to endogenous IFN in vivo may lead to a decline in the oxidative metabolism of the granulocytes, which gives a reduced capacity of the host to defend itself against bacterial superinfection. Until further investigations on the functions of granulocytes during respiratory tract viral infections have been performed, the role of IFN in the pathogenesis of these diseases remains uncertain.

Treatment of patients with human IFN-α did not have any major effect on the phagocytic activity of granulocytes (Table 2). When granulocytes are incubated with IFN in vitro, their phagocytic activity increases (15a). The reason for the lack of effect in vivo is not known. One possibility is that the phagocytic activity of granulocytes increased at a certain stage of treatment, although we failed to detect it. Thereafter, the neutrophils are "worn out" by the continuous presence of IFN in vivo. Another possibility is that factors functioning only in vivo counteract the stimulating effect of IFN. We have previously observed that the phagocytic activity of blood monocytes decreases during long-term treatment with IFN-α (8), although this function is augmented after treatment with IFN in vitro (13, 15).

The capacity of the patient granulocytes to reduce NBT in the absence of bacterial stimuli and autologous plasma was lower than that of granulocytes from healthy donors (Table 1). Our patient group is relatively small, and further studies, with age- and sex-matched donors, have to be performed before more definite conclusions can be drawn in regard to differences in the NBT-reducing capacity of granulocytes from tumor patients and healthy donors. In this context it is of interest to note that the cytostatic activity of granulocytes against a lymphoblastoid cell line has been reported to be lower in tumor patients than in healthy donors (16).

IFN is produced during the course of various diseases, and at several clinics, exogenous IFN is administered to patients. It is therefore of importance to learn more about the in vivo influence of IFN on cellular functions. In viral and bacterial infections, granulocytes are known to participate in the defense of the host against the disease, and this has also been suggested to be the case for tumor diseases (16). This study indicates that the oxidative metabolism of blood granulocytes is affected by IFN in vivo. Whether this has relevance for the capacity of the immune system to defend the host against bacterial and viral infections can only be speculated on.

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