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Infectious Complications and Host Immune Defense in Acute Leukemia

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Abstract—*Microbiological and immunological studies were carried out on 73 acute leukemia patients in order to establish the relationship between the host immune defense and the frequency and severity of infections. Pronounced disturbances in the functional activity of granulocytes, the total complement hemolytic activity and C_3 component of the complement are found during induction chemotherapy; in this period the infectious complications are more frequent and severe than at the onset of the disease. No relationship is established between serum lysozyme levels and resistance to infections. The elevated immunoglobulins G and M as a possible response to the infectious agents are considered. It is emphasized that a pronounced immunosuppression as well as the most frequent infectious complications are found in patients in relapse.*

INTRODUCTION

INFECTIONS are the most frequent complication and the leading cause of death in patients with acute leukemia. This fact is supported by observations made by a number of authors [1–9]. The enhanced risk of infectious complications may be due both to the basic neoplastic disease, involving the cellular compartment of the immune system, and to the immunosuppressive effect of contemporary intensive chemotherapy. The close relationship between the frequency of infections and granulocytopenia in treated leukemic patients has already been pointed out [10, 11]. It is also well known that these patients demonstrate substantial changes in the phagocytic and bactericidal activity of granulocytes [12–16], serum immunoglobulin levels [17–19], lysozyme [20–25] and some cell-mediated immune reactions [17, 26–30].

The object of this paper is to present the relationship between the frequency and severity of infections and the host defense in acute leukemia patients. It reflects the results of current studies of some of the most important factors of the immune system along with clinical and laboratory investigations on the infectious process.

MATERIALS AND METHODS

Patients

Studies have been carried out on 73 acute leukemia patients aged between 16 and 78, who have undergone regular clinical, microbiological and immunological investigations throughout the course of the disease.

Clinical and microbiological investigations

On the day of admission patients were subjected to a thorough clinical examination. Microbiological cultures were made of oral secretions, sputum, urine, material from manifested infectious loci and hemoculture, the last in cases of fever. During induction chemotherapy the analyses were repeated depending upon the clinical and hematological evolution of the disease. Routine methods of microbiological diagnosis were used [31].

Immunological studies

On the day of admission, during induction chemotherapy and at diagnosis of infectious complications a close observation was maintained over the absolute granulocyte count in the peripheral blood, the phagocytic activity against *Staph. aureus*, the ability of granulocytes to ingest and kill *Candida albicans*, the serum immunoglobulin and lysozyme levels, and the total complement hemolytic activity and C_3 component of complement.

The phagocytic function of peripheral blood granulocytes against *Staph. aureus* was tested according to the method of Van Furth [32], with determination of the indices phagocytic activity (PhA) and phagocytic number (PhN). The PhA represents the percentage of granulocytes which have ingested *Staph. aureus* and the PhN represents the average number of

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Staph. aureus ingested by one phagocyte. For the quantitative assessment of the phagocytic efficiency two other indices were computated, i.e. phagocytic capacity (PhC) and phagocytic capacity index (PhCI). The first reflects the number of microorganisms ingested by the granulocytes in 1 μl peripheral blood, and is determined by the following formula:

$$\text{PhC} - \frac{A \times B \times C}{100},$$

where A = number of granulocytes per microliter blood, B = PhA, and C = PhN, while

$$\text{PhCI} - \frac{\text{PhC of the patient}}{\text{PhC of healthy subjects}}$$

represents the ratio of PhC of acute leukemia patients and that of healthy subjects.

The bactericidal activity of granulocytes against *Candida albicans* was assayed by the method of Lehrer and Cline [14].

The total complement hemolytic activity was determined according to the method of Kabat and Mayer [33].

The C_3 *component of the complement* was assayed by the single radial immunodiffusion method of Mancini [34].

Serum lysozyme levels were determined by the lysoplate method of Osserman and Lawlor [35].

The serum immunoglobulins G, A and M were determined by the method of Mancini [34].

Statistical analysis.

Computer aided calculation of the numerical characteristics of the observed factors is given, based on the analysis of variance. Authors have developed FORTRAN IV software of the problem, which can be successfully applied by other users.

RESULTS

Infectious complications. Frequency, sites and isolated microorganisms

Of the total of 60 hospitalized patients in the initial stage of the disease, 35 (58.33%) were febrile; the febrility in 19 was accompanied by clinical signs of infection. Significant pathogens from various infectious sources were isolated in 24 patients (40%); in some of them there was more than one infection, so that the total number of infectious episodes was 44, or 0.73 infections per patient (Fig. 1). The sites of infections in the various stages of the disease are tabulated in Table 1. Severe infections such as sepsis and disseminated infections (three or more per patient)

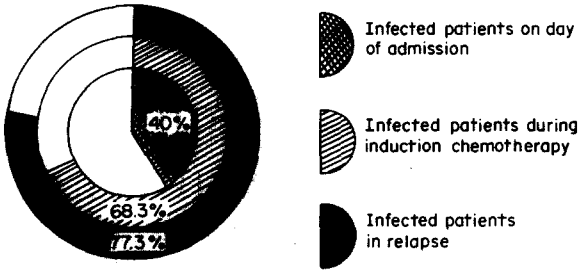

Fig. 1. Frequency of infectious complications in acute leukemia patients.

Table 1. Sites of infections in acute leukemia patients

Site	Number of patients with sites: On admission	During induction chemotherapy	In relapse
Lungs	14	30	11
Oral cavity	14	27	15
Genito-urinary tract	9	17	10
Skin	3	7	4
Nose	—	3	1
Meninges	—	—	1
Septicemia	4	7	4
Total sites	44	95	46
Infected patients	24/60	41/60	17/22

were established in 6.6% and 3.3%, respectively. The most commonly isolated pathogens are recorded in Table 2.

During induction chemotherapy clinically manifested and bacteriologically confirmed infections were noted in 17 of the patients not infected at admission to hospital; 8 of initially infected patients developed new sites of infection. The overall number of infected patients was 41 (68.33%); the number of infectious episodes was 95 or 1.52 infections per patient. The sites of infections and isolated pathogens remained approximately the same as in the initial stage of acute leukemia. Frequency of sepsis and disseminated infection displayed a marked increase.

Infections were documented in 17 (77.3%) of the 32 patients hospitalized in relapse, with a total of 46 infectious episodes, or 2.1 infections per patient. A further increase in the frequency of septicemia cases was noted. A case of Gram negative Pseudomonas meningitis deserves to be mentioned.

Immunological status

The absolute granulocyte count in the peripheral blood and the PhCI are presented in Table 3.

Table. 2. Pathogens isolated from microbiologically documented infections in acute leukemia patients

Pathogens	On day of admission		During induction chemotherapy		In relapse	
	No	%	No	%	No	%
Gram negative bacteria	47	74.6	79	65.3	20	47.6
Klebsiella spp.	21	44.7	37	46.8	6	30
Escherichia coli	13	27.7	24	30.4	9	45
Pseudomonas aerug.	8	17.0	9	11.4	2	10.0
Proteus spp.	5	10.6	8	10.1	3	15.0
Alcaligenes faec.	—	—	1	1.3	—	—
Staphylococcus aureus	7	11.1	21	17.4	16	38.1
Candida	9	14.3	20	16.5	6	14.3
Streptococcus faecalis	—	—	1	0.8	—	—
Total	63		121		42	

Table 3. Absolute granulocyte count and PhCI (Mean values ± S.D.) in acute leukemia patients

Index	On day of admission		During induction chemotherapy		Patients with severe infections	Infected patients in relapse	Healthy controls
	A*	B	A	B			
Absolute granulocyte count	2541 ±2310	1018 ±637 $P<0.05$	886 ±172 $P<0.05$	427 ±225 $P<0.05$	291 ±180 $P<0.05$	345 ±250 $P<0.05$	3500 ±300
Phagocytic capacity index	1 ±0.62	0.65 ±0.33	0.45 ±0.17 $P<0.05$	0.20 ±0.11 $P<0.05$	0.13 ±0.07 $P<0.05$	0.16 ±0.12 $P<0.05$	1

*A, Patients without infections; B, patients with infections.

In acute leukemia patients the indices of the phagocytic process displayed different changes. Thus, in the initial stage, along with neutropenia, a certain stimulation of the PhA as well as of the PhN occurred, while during induction chemotherapy and the further decrease of the granulocyte count a substantial decrease in those indices was observed. On this ground we assumed the index PhC to be more informative, since it simultaneously reflects the changes occurring in the three components of phagocytosis: the absolute granulocyte count, the PhA and the PhN. For the sake of convenience the resulting values are presented by the PhCI. Thus, in the initial stage of acute leukemia we found a lower PhCI in infected patients. During intensive chemotherapy its values decreased in patients of both groups. The lowest values were observed in patients with severe infections and in relapse.

The bactericidal activity of granulocytes, expressed as a percentage of killed *Candida*, is presented in Fig. 2. The granulocytes of all

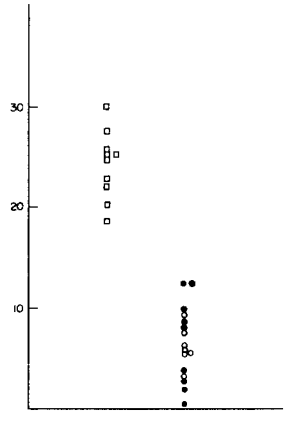

Fig. 2. Bactericidal activity of granulocytes in acute leukemia patients and healthy controls. □ Healthy controls, ● acute leukemia patients infectious complications during induction chemotherapy, ○ acute leukemia patients without infectious complications during induction chemotherapy.

acute leukemia patients examined during induction chemotherapy demonstrated a strongly inhibited bactericidal activity.

Complement. The mean values of the total complement hemolytic activity (CH_{50}) and the

C_3 component in acute leukemia patients are presented in Fig. 3. A decreased complement activity was established in infected patients during induction chemotherapy, particularly in patients with severe infections and in relapse. The C_3 component levels displayed variations similar to those for CH_{50}.

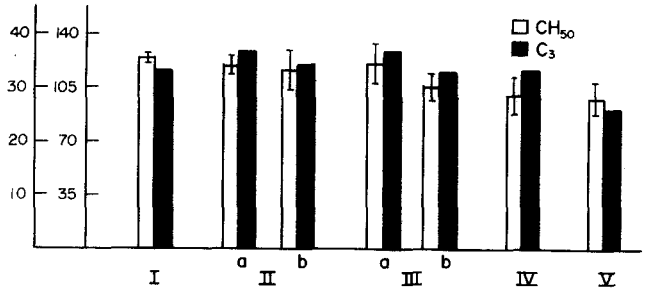

Fig. 3. Total complement hemolytic activity and C_3 component in acute leukemia patients and healthy controls. I, *Healthy controls;* II, *acute leukemia patients on day of admission;* III, *acute leukemia patients during induction chemotherapy; IV, Acute leukemia patients in relapse;* V, *acute leukemia patients with severe infections.* a, *Patients without infections;* b, *patients with infections.*

Lysozyme. Acute leukemia patients display variations in serum lysozyme values primarily depending upon the form of the disease. A quantitative increase of over 100 mcg/ml was observed in acute monoblastic leukemia, while acute lymphoblastic and acute myeloblastic leukemia patients had comparatively low levels during induction chemotherapy. No relationship was observed between lysozyme values and frequency and severity of infectious complications. A significant decrease was established only in infected patients in relapse—4.75 mcg/ml ($P<0.001$).

Immunoglobulins. Serum immunoglobulin G, A and M levels in acute leukemia patients and healthy controls are presented in Table 4. Despite the great individual variations in all three classes significantly increased IgM values were established in patients with infectious complications in the initial stage of the disease. An increase of IgG was observed in some infected patients during induction chemotherapy. Decreased levels of all three Ig classes were established in patients in relapse.

DISCUSSION

The results demonstrate a dependence between host defense of acute leukemia patients and the occurence of infectious complications, the frequency and severity of which increases with the aggravation of immunosuppression. Particularly marked is the participation of granulocytes in the anti-infectious resistance in all stages of acute leukemia, where not only their absolute count, but also their functional activity is of substantial importance. At the onset of the disease granulocytopenia does not seem to be obligatory for all patients; their phagocytic function is preserved or even compensatorily increased, as has been reported by other authors also [36]. No particular changes

Table 4. Immunoglobulin G, A and M levels (mean values ± S.D.) in acute leukemia patients

Immunoglobulin (mg%)	On day of admission		During induction chemotherapy		Patients with severe infections	Infected patients in relapse	Healthy controls
	A*	B	A	B			
IgG	1230 ±389	1289 ±258	1474 ±510	880 ±255	918 ±303	797 ±779 $P<0.05$	1131 ±189
IgA	220 ±55	207 ±153	184 ±51	164 ±63	182 ±47	154 ±99	181 ±30
IgM	118 ±42	143 ±28 $P<0.05$	85 ±60	124 ±46	138 ±23	59 ±49 $P<0.05$	110 ±19

*A, Patients without infections; B, patients with infections.

in humoral factors were observed. In this period infections are less frequent and they arise mainly in granulocytopenic patients in whom the cellular antibacterial defense is inhibited by quantitative changes in granulocytes. The increased IgM values in infected patients may be considered as an expression of an early humoral immune response against the pathogenic agents.

During induction chemotherapy marked granulocytopenia is established in all patients, accompanied by functional disorders in the granulocytes. These changes lead to a strongly reduced phagocytic capacity. The bactericidal activity of granulocytes is also suppressed, most probably in connection with an impaired metabolism and enzyme synthesis [13, 15, 16]. Consequently, with the suppressed cellular antibacterial resistance, the frequency of infectious complications is augmented, as well as the percentage of patients with severe infections. The increased IgG levels during that period in some of the infected patients most probably reflects the synthesis of antibodies against bacterial antigens. High levels of immunoglobulins during the active stage of acute leukemia is reported by other authors as well [19, 36]. However, these authors do not follow the association of immunoglobulins with the infections concomitant to the basic disorder. The decreased complement activity of the serum may be explained first of all by the suppressed synthesis of the complement components as a result of the administered cytostatic agents or of a leukemic infiltration of the reticuloendothelial organs. It is highly probable also that its cause lies in the consumption of complement in the course of bacteriolysis, or in its utilization along the alternative pathway by the lipopolysaccharides of Gram negative micro-organisms. Both in that period of the disease and in the preceding one no persistent correlation between serum lysozyme levels and the frequency and severity of bacterial infections has been established.

The most pronounced immunosuppression is observed in patients in relapse, most probably in connection with previous courses of more or less prolonged chemotherapy. Parallel to the quantitative and functional damage of the cellular granulocytic antibacterial defense there occurs a reduction of all humoral immune factors. Infections are most frequent in this stage, with the highest percentage of sepsis and disseminated infection.

The relationship between factors of host immune defense and infectious complications in acute leukemia patients clearly reveals the significance both of cellular and humoral immunity. Granulocyte function undergoes the earliest and most profound changes, while the humoral factors become affected later, during intensive induction chemotherapy and particularly in relapse, when one may witness a complete failure of host immune defense. Those facts should be remembered in the prophylaxis and management of infectious complications in acute leukemia.

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