Defective neutrophil chemotaxis in bone marrow transplant patients.

R A Clark, … , S J Klebanoff, E D Thomas

Research Article

Infection is a frequent cause of death in patients receiving bone marrow transplants. Although lymphocyte dysfunction has been observed in a few such patients, no systematic study of neutrophil function has yet been reported. Neutrophil chemotaxis was evaluated by a 51Cr-radioassay after bone marrow transplantation in 34 patients with acute leukemia or aplastic anemia. The response to a chemotactic stimulus (C5a) was severely depressed (less than 35% of normal) in 18 patients, moderately depressed (35-65% of normal) in an additional 6, and normal in 10 subjects. The mean response in the absence of graft vs. host disease and antithymocyte globulin administration was 73.3+/-9.2% (SE) in contrast to 29.7+/-9.6% (P is less than 0.01) in patients with graft vs. host disease treated with antithymocyte golbulin. Both graft vs. host disease and antithymocyte globulin were implicated since the presence of either factor alone was associated with depressed chemotaxis (31.1+/-4.9% for graft vs. host disease, P is less than 0.01; 17.0+/-7.8% for antithymocyte globulin, P is less than 0.02). When normal neutrophils were incubated with antithymocyte globulin in vitro, their chemotactic response was markedly suppressed in the absence of a cytotoxic effect. Transplant patients with defective chemotaxis experienced significantly more infections than those with normal chemotaxis, and analysis of specific etiologic agents showed that this was predominantly related to bacterial pathogens. Chemotactic inhibitors […]

Find the latest version:

Defective Neutrophil Chemotaxis in Bone Marrow Transplant Patients

ROBERT A. CLARK, F. LEONARD JOHNSON, SEYMOUR J. KLEBANOFF, and
E. DONNALL THOMAS

*From the Department of Medicine, University of Washington School of Medicine,
Seattle, Washington 98195*

A B S T R A C T Infection is a frequent cause of death in
patients receiving bone marrow transplants. Although lym-
phocyte dysfunction has been observed in a few such pa-
tients, no systematic study of neutrophil function has
yet been reported. Neutrophil chemotaxis was evaluated
by a ^{51}Cr-radioassay after bone marrow transplantation
in 34 patients with acute leukemia or aplastic anemia.
The response to a chemotactic stimulus (C5a) was se-
verely depressed ($< 35\%$ of normal) in 18 patients,
moderately depressed (35–65% of normal) in an addi-
tional 6, and normal in 10 subjects. The mean response
in the absence of graft vs. host disease and antithymo-
cyte globulin administration was 73.3±9.2% (SE) in
contrast to 29.7±9.6% ($P < 0.01$) in patients with graft
vs. host disease treated with antithymocyte globulin.
Both graft vs. host disease and antithymocyte globulin
were implicated since the presence of either factor alone
was associated with depressed chemotaxis (31.1±4.9%
for graft vs. host disease, $P < 0.01$; 17.0±7.8% for anti-
thymocyte globulin, $P < 0.02$). When normal neutro-
phils were incubated with antithymocyte globulin in
vitro, their chemotactic response was markedly sup-
pressed in the absence of a cytotoxic effect. Transplant
patients with defective chemotaxis experienced signifi-
cantly more infections than those with normal chemo-
taxis, and analysis of specific etiologic agents showed
that this was predominantly related to bacterial patho-
gens. Chemotactic inhibitors were detected in the sera
of seven patients and elevated IgE levels were found in
nine subjects, eight of whom had graft vs. host disease.
Generation of chemotactic activity by endotoxin acti-

vation of serum was reduced in five patients. The re-
sults demonstrate a severe defect in neutrophil chemo-
taxis in some bone marrow transplant patients and sug-
gest that neutrophil dysfunction may predispose to in-
fection in such patients.

INTRODUCTION

Bone marrow transplantation is being employed with
increasing frequency in the management of acute leuke-
mia and aplastic anemia (for review see 1). Although
steady improvement in results has been achieved, a
number of problems continue to limit long-term suc-
cess. Among the most frequent causes of morbidity and
mortality in these patients are graft vs. host disease
(GVHD)[1] (2) and severe infections with a variety of
bacterial, viral, fungal, and protozoal organisms (2–4).

The factors responsible for the high incidence of in-
fections are poorly understood at present. During the
early postgrafting period, granulocytopenia is undoubt-
edly a major factor, since successful engraftment as de-
fined by an absolute granulocyte count of 500/mm^3 or
greater does not usually occur for 2–4 wk (1). The use
of immunosuppressive drugs (1) may also increase sus-
ceptibility to infection. A marked increase in mortality
from infection has been noted in marrow transplant
patients who develop severe GVHD (2), suggesting the
possibility of compromised host defense mechanisms in
this group in particular. Studies of both human and ca-
nine marrow graft recipients have demonstrated de-
creased immunologic responsiveness which is particu-
larly prominent in the early postgrafting period (5–8).
Granulocyte function has not been systematically evalu-
ated in bone marrow transplant patients, although nor-

---

[1] *Abbreviations used in this paper:* ATG, antithymocyte
globulin; GVHD, graft vs. host disease; PMN, polymorpho-
nuclear leukocyte.

mal granulocyte iodination and nitroblue tetrazolium reduction were reported in canine marrow graft recipients (7).

In an attempt to determine whether granulocyte dysfunction may contribute to the increased susceptibility to infection in marrow transplant patients, polymorphonuclear leukocyte (PMN) chemotaxis was evaluated after 34 marrow grafts in patients with leukemia or aplastic anemia. A marked decrease in chemotactic responses was observed in a large proportion of these patients. The relationship of this defect to a number of clinical parameters including GVHD and the administration of antithymocyte globulin (ATG) was also examined.

METHODS

Study population. Between November 1973 and August 1975, studies were performed after 34 marrow transplants (see Table I) in 33 patients, 1 individual receiving a repeat graft after recurrence of leukemia (see Table I, numbers 5 and 29). 12 patients had aplastic anemia, 10 had acute lymphocytic leukemia, 10 had acute myelocytic leukemia, and 1 had chronic myelocytic leukemia in blast crisis. The patients' ages ranged from 2 to 49 yr (mean 19) and 19 were males. Transplants were performed on 1 patient in 1971, 3 in 1973, 20 in 1974, and 10 in 1975. Patients were studied from 22 to 1,149 days after transplantation, with a median of 66 days. Serial studies of chemotaxis were performed in 14 patients, and a total of 67 studies were done in the entire group of 33 patients.

TABLE I
PMN Chemotactic Responses

Patient*	Age	Sex	Diag-nosis‡	Study day§	GVHD‖	Days since ATG¶	PMN chemo-taxis**	Patient*	Age	Sex	Diag-nosis‡	Study day§	GVHD‖	Days since ATG¶	PMN chemo-taxis**
Group A								21	29	M	AML	136	0	—	42
1	16	F	AA	86	III	29	2					199	0	—	59
2	6	F	AA	49	0	53	3	22	21	M	ALL	72	0	—	53
3	27	M.	AA	64	III	10	5					177	0	—	51
4	2	M	AML	92	III	—	6	23	9	M	ALL	406	IIIC	—	34
5	20	F	ALL	25	III	2	4					413	IIIC	—	74
				31	III	3	8					414	IIIC	—	49
6	3	M	AA	24	I	—	7					421	IIIC	—	35
7	31	F	AML	42	III	2	11					426	IIIC	—	75
8	49	M	AML	52	IV	3	13					431	IIIC	—	37
9	8	M	AML	42	III	8	18					431	IIIC	—	24
10	30	M	AML	52	IV	—	31					440	IIIC	—	94
				80	IV	18	5					905	IIIC	—	62
11	4	F	AA	22	0	26	18					919	IIIC	—	48
12	13	F	AA	42	II	3	21	24	16	M	AA	827	0	—	42
13	17	M	AA	49	II	—	22					864	0	—	49
				57	III	1	7					906	0	—	41
				91	III	30	33					934	0	—	94
				238	IVC	116	28					990	0	—	121
14	11	M	ALL	71	II	32	22					1,025	0	—	47
15	30	M	AML	87	III	51	41					1,149	0	—	75
				136	III	100	15	**Group C**							
				143	III	107	19	25	9	M	ALL	22	0	—	65
16	9	F	AML	80	I	15	30	26	15	M	ALL	41	I	—	67
17	32	F	AA	42	II	—	30	27	8	F	ALL	31	0	—	71
				84	III	3	31	28	30	F	AA	64	0	—	98
18	49	M	AML	49	III	10	31					92	0	—	70
Group B								29	20	F	ALL	511	0	>400	102
19	10	M	ALL	110	0	—	37	30	5	F	ALL	62	III	29	104
				161	0	—	42					90	III	47	107
				407	IIIC	—	39	31	30	F	AML	49	I	—	110
20	35	M	AA	388	IIIC	>200	51	32	14	F	AA	30	0	—	118
				396	IIIC	—	20	33	18	F	CML, BC	52	I	—	100
				437	IIIC	—	40					178	0	—	140
				563	IIIC	—	51	34	4	M	ALL	87	II	4	129

* Patients indicated by number in order of increasing average chemotactic response. Patient 5 and 29 are the same individual after two separate marrow transplants (see Methods). The consecutive numbers used for convenience in this study correspond to the unique patient identification numbers cited in other publications from this center as follows: 1 = 374, 2 = 503, 3 = 341, 4 = 338, 5 = 272, 6 = 512, 7 = 506, 8 = 358, 9 = 331, 10 = 392, 11 = 399, 12 = 394, 13 = 330, 14 = 500, 15 = 377, 16 = 386, 17 = 366, 18 = 393, 19 = 329, 20 = 294, 21 = 325, 22 = 334, 23 = 274, 24 = 114, 25 = 359, 26 = 347, 27 = 339, 28 = 335, 29 = 272, 30 = 350, 31 = 353, 32 = 349, 33 = 336, and 34 = 365.
‡ AA, aplastic anemia; AML, acute myelocytic leukemia; ALL, acute lymphocytic leukemia; CML, BC, chronic myelocytic leukemia in blast crisis.
§ Days after transplantation.
‖ Graft vs. host disease graded 0, I, II, III, and IV as described (2, 13). C indicates chronic GVHD.
¶ Days since most recent administration of antithymocyte globulin; dash (—) indicates no ATG administered.
** Chemotaxis data expressed as percent of concomitantly studied normal control.

Patient management. The technique for performing bone marrow transplantation has been described in detail (1, 9–11). Transplants were done between HLA identical siblings whose lymphocytes were nonreactive in mixed leukocyte culture, or in one instance (Table I, number 27) between identical twins. Pregraft preparation in patients with leukemia consisted of cyclophosphamide and total body irradiation (1, 10). Most patients with aplastic anemia were prepared with cyclophosphamide alone (1, 11), but four (Table I, numbers 1, 2, 3, and 11) received in addition procarbazine and ATG (12). After marrow infusion patients were supported with platelet and erythrocyte transfusions, and granulocyte transfusions were given in some instances for treatment of bacterial infection occurring before successful engraftment. All patients except the identical twin received methotrexate 15 mg/M₂ on day 1 after transplantation and then 10 mg/M₂ on day 3, 6, 11, and weekly until day 100.

Many patients developed some manifestations of GVHD during the post-transplantation course. This was graded in severity from I to IV on the basis of previously defined clinical and histologic criteria (2, 13). GVHD persisting for more than 3 mo or developing beyond 100 days posttransplantation was termed chronic GVHD (2). Most patients with Grade II–IV and one patient with Grade I GVHD were treated with ATG raised in rabbits, goats, or horses and given in a dose of 7 mg/kg every other day for six doses as previously described (14). For purposes of analysis, patients with no evidence of GVHD were grouped together with those having minimal disease with skin involvement only (Grade I) and compared to those having more advanced disease (Grade II–IV) as in previous reports (2). Most patients experienced one or more episodes of infection during the postgrafting period. These were treated aggressively with appropriate antimicrobial agents and supportive care. Chemotaxis was studied predominantly during noninfected periods. Some subjects were studied during active infection, although none was severely ill with sepsis or shock. Patient 2 had bacteremia 2 days before and again 5 days after study. During the course of the study, 18 patients died at an average of 139 days (range 29–370, median 92) after transplantation or 80 days (range 3–298, median 38) after evaluation of chemotaxis. The average follow-up period in the 15 patients who are still alive was 581 days (range 180–1,584, median 484) after transplantation or 428 days (range 156–757, median 435) after evaluation of chemotaxis.

Chemotaxis technique. PMN chemotaxis was evaluated by a previously described in vitro method employing ⁵¹Cr-labeled neutrophils and a two-filter Boyden chamber system (15) which will be summarized briefly. Leukocytes were harvested from heparinized blood by dextran sedimentation and hypotonic lysis of erythrocytes. The washed leukocytes were labeled with Na₂ ⁵¹CrO₄ (New England Nuclear, Boston, Mass., 200–500 μCi/μg Cr), washed and suspended in Gey's medium (Microbiological Associates, Bethesda, Md.) at 2.3×10^6 PMNs/ml (purity 80–90% granulocytes). PMN suspensions were placed in the upper compartment of standard plexiglass Boyden chemotaxis chambers (Ahlco Scientific, Granby, Conn.) and were separated from the chemotactic stimulus in the lower compartment by two nitrocellulose micropore filters with an average pore size of 3 μm (Sartorius Membranfilter, Göttingen, W. Germany). After a 3-h incubation period, the amount of radioactivity in the lower filter was determined by counting in a Packard Auto-Gamma scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). It has been shown that under these conditions only granulocytes migrate into the lower filter (15). After adjusting for variability in specific activity and granulocyte uptake of ⁵¹Cr, chemotaxis was expressed as corrected counts per minute in the lower filter (15).

The chemotactic stimuli employed were (a) partially purified human C5a generated by endotoxin activation of serum, eluted from Sephadex G-75 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and characterized by heat stability, molecular weight (15,000), and inhibition by goat antihuman C5 (16) and (b) endotoxin (*Escherichia coli* 0127:B8 lipopolysaccharide B, Difco Laboratories, Detroit, Mich.) activated whole human serum (17). Patients' cells and serum were always studied concomitantly with cells and serum from one or more normal individuals. For nine of the patients (Table I, numbers 5, 13, 19, 20, 22, 24, 27, 28, and 29), the bone marrow graft donor sibling was employed for a normal control, while in the remainder healthy laboratory personnel of both sexes ranging in age from 18 to 48 yr were used. According to the experimental protocol normal and patient PMNs were tested against a buffer negative control (gelatin veronal buffer) (17), C5a, and endotoxin activated normal and patient serum. Each condition was examined in quadruplicate chemotaxis chambers, and the mean response of patient cells expressed as a percent of the mean response of normal cells. The chemotactic activity of patient serum was compared to normal serum in similar fashion.

The possibility of chemotactic inhibitors in the patient's serum was evaluated by comparing the response of normal PMNs suspended in Gey's medium containing 10% patient serum to the response of the same normal PMNs suspended in Gey's medium containing 10% normal serum (17). Serum immunoglobulin E levels were measured by radioimmunoassay in a commercial laboratory (Bio-Science Laboratories, Van Nuys, Calif.). All other hematological, chemical, microbiological, and histological studies were performed by standard techniques in the hospital diagnostic laboratories.

The in vitro effect of ATG on chemotaxis was examined by suspending normal neutrophils in Gey's medium containing various dilutions of ATG. After a 10-min pre-incubation at 37°C, these suspensions were placed in chemotaxis chambers and their response to C5a determined. In experiments designed to differentiate between a direct effect of ATG on the neutrophil as opposed to an effect on the chemotactic factor, the PMN suspensions were centrifuged, washed free of ATG, and resuspended in fresh Gey's medium before placement in the chemotaxis chambers. Neutrophil cytotoxic activity of ATG was evaluated by incubating suspensions of highly purified (Ficoll-Hypaque gradient method, see 15, 17) normal ⁵¹Cr-labeled neutrophils (2.3×10^6 PMNs/ml) in Gey's medium containing various dilutions of ATG for 60 min at 37°C. After centrifugation (300 g for 10 min), release of ⁵¹Cr was determined by measuring counts per minute in the supernates and comparing the experimental values with negative (no ATG) and positive (1% Triton X-100, Sigma Chemical Co., St. Louis, Mo.) controls. ATG was prepared in rabbits (Batch 17) and goats (Batch 8) as described (14); horse ATG (Lot 17,900) was obtained through the courtesy of the Upjohn Co., Kalamazoo, Mich. Protein concentration was comparable in the three lots (approximately 50 mg/ml), and potent immunosuppressive activity was confirmed by assays of lymphocytotoxicity, hemagglutination, and monkey skin graft survival (14).

Statistics. Standard error was used throughout as an estimate of variance and means were compared by use of the *t* test. As reported previously (15), the variability in

the chemotaxis technique is such that the use of quadruplicate chambers results in standard errors which average approximately 10% of the mean. Values of less than 65–70% of the normal control were significantly reduced in nearly all instances, while those above this level were not. Nonparametric data were compared by the chi-square method or the Fisher exact test.

RESULTS

PMN chemotactic responses. Table I lists the patients and their chemotaxis results in order of increasing average PMN chemotactic response. Selected clinical parameters are also tabulated. For purposes of analysis the patients are divided into three groups: Group A (patients 1–18), PMN chemotactic response severely depressed ($< 35\%$ of normal); Group B (patients 19–24), PMN chemotactic responses moderately depressed (35–65% of normal); and Group C (patients 25–34), PMN chemotaxis not significantly reduced ($\geq 65\%$ of normal). The values for patients in Groups A and B were significantly reduced from normal (with $P < 0.01$ in most instances), except for 1 of 10 studies in patient 23 and 3 of 7 studies in patient 24 with P values > 0.05. Thus, defects in the PMN chemotactic response to C5a were observed in approximately three-fourths (24 of 34) of all the bone marrow transplant recipients studied.

As detailed in Table I, 14 patients were studied on more than one occasion. Findings remained relatively constant with the exception of isolated patients whose clinical features changed dramatically between studies. Some of these are discussed in a later section as they relate to GVHD and therapy with ATG. Little change was noted in patients 15, 20, 21, 22, 28, 30, and 33. Patient 23 was studied on 10 separate occasions, with significantly reduced migration being observed in all but one experiment. In patient 24 the first three studies were performed during a period of 3 mo when he experienced two bouts of bacterial pneumonia (*Streptococcus pneumoniae* and *Hemophilus influenzae*); all showed decreased chemotactic responses. Subsequently, the chemotaxis values increased to normal levels, and infections ceased to be a problem.

Correlation of chemotaxis with clinical parameters. A number of important clinical features of the patients were examined to determine which factors might be associated with the depressed PMN chemotactic response. There was a tendency for better PMN chemotactic responses in patients with acute lymphocytic leukemia relative to those with either aplastic anemia ($0.05 < P < 0.1$) or acute myelocytic leukemia ($P < 0.05$). Age did not appear to be a determinant of the chemotactic response since no significant differences were noted among Groups A, B, and C (mean age 19.8±3.5, 20.0±4.3, and 15.5±3.1 yr, respectively). Females relatively outnumbered males in the normal chemotaxis group, but the average response of all female patients did not differ significantly from that of the male patients. Elapsed time since marrow infusion was comparable in Groups A and C (median 58 and 68 days, respectively), although an apparently chance accumulation of longer term patients was noted in Group B.

Absolute granulocyte counts at the time of study varied widely. Patients with very low counts due to delayed engraftment could not be studied. Only five subjects (numbers 2, 3, 4, 7, and 11) had values less than 1,000/mm³, while two had markedly elevated values (17,800 and 20,930 in patients 8 and 30, respectively). The mean absolute granulocyte count was lower in Group A (3,132±930) than in either Group B (5,015±1,265) or C (5,358±1,783), but with the wide variability this did not prove to be significant. Abnormal liver function tests (serum bilirubin and hepatic enzymes) were observed more frequently in Groups A and B than in Group C, primarily because these abnormalities were present in patients with GVHD (see below). Tests of renal function (blood urea nitrogen and serum creatinine) were normal in all subjects.

Serum IgE levels were determined since a number of patients with recurrent infections, defective PMN chemotaxis, and hyperimmunoglobulinemia E have been reported (18–22). Normal values (< 300 U/ml) were observed in most patients, but elevated levels were present in nine individuals (six in Group A, two in Group B, and one in Group C). In six of these, values were between 600 and 900 U/ml, but more marked elevations were noted in patients 4, 5, and 15 (1,050, 3,379, and 1,051 U/ml, respectively), all of whom had severely depressed chemotaxis. The hyperimmunoglobulinemia E may be related to GVHD since eight of the nine patients with elevated levels had Grade III-IV GVHD and three of four patients with chronic GVHD had high IgE levels.

The possible contribution of drugs to the chemotaxis results was examined carefully. All patients studied within 100 days of marrow infusion (except for the 1 identical twin, patient 27) were receiving weekly methotrexate; this included 17 of 24 with decreased PMN chemotaxis (Groups A and B) and 8 of 10 with normal chemotaxis (not significant). The 10 patients receiving prednisone (2.5–60 mg daily, mean 30 mg) were evenly distributed in Groups A, B, and C (3, 3, and 4 patients, respectively). 19 patients were receiving one or more systemic antimicrobial agents at the time of at least one chemotaxis study; these drugs included cephalosporins, gentamicin, carbenicillin, penicillins, amphotericin B, and pentamidine isethionate. PMN chemotactic responses did not correlate with administration of any of these agents.

In analyzing the relationship between chemotactic responses and the occurrence of infections, only well-documented infections developing in spite of an absolute

granulocyte count greater than 1,000/mm³ were considered. Specific infections are tabulated for the different patient groups in Table II. Infections occurred in 22 of 24 patients with defective chemotaxis (Groups A and B) and in 6 of 10 with normal chemotaxis ($P = 0.043$, Fisher exact test). It is evident from Table II that many patients experienced multiple infections with a total of 70 distinct episodes in all. The mean number of infections per patient was 2.46 in Group A and B combined and only 1.10 in Group C ($P < 0.05$, t test). Bacterial infections were observed in 16 of the 24 Group A and B patients and in 3 of 10 Group C patients ($P = 0.048$, Fisher exact test). Bacterial infections per patient averaged 1.54 in patients with diminished chemotaxis (Groups A and B combined) as opposed to only 0.30 in those with normal chemotaxis ($P < 0.05$, t test). Although septicemia was also much more frequent in the 24 Group A and B patients (14 episodes in 10 patients) than in the 10 Group C patients (1 episode), this difference did not prove to be significant ($P = 0.068$, Fisher exact test; $0.05 < P < 0.1$, t test). Bacterial pneumonia (10 episodes in six patients) and meningitis (2 episodes)

occurred exclusively in patients with depressed chemotactic responses. A total of 20 cases of bilateral interstitial pneumonia (3) were observed, 5 due to cytomegalovirus, 2 due to varicella-zoster, 4 due to *Pneumocystis carinii,* and 9 of unproven etiology. These 20 infections occurred in 13 Group A and B patients (14 infections) and 5 Group C patients (6 infections), (difference not significant). Of 13 proven viral infections, 10 were seen in 8 different Group A and B patients and 3 in Group C patients (not significant). All four cases of disseminated fungal infection occurred in Group A and B patients, although this was not a statistically significant finding. The differences in infections described could not be attributed to differences in follow-up, since the period of observation was similar in all groups (see Methods). Death due to infection (mostly interstitial pneumonia) occurred in 13 patients with depressed chemotaxis and in 4 patients with normal chemotaxis. Thus, defective chemotaxis in these patients was associated with an increased incidence of serious infections caused predominantly by bacterial pathogens. Fungi may possibly be implicated as well, but the incidence of viral

TABLE II
Infections Relative to Chemotactic Responses

| | Patients* | | |
Type of infection	Group A (1 to 18)	Group B (19 to 24)	Group C (25 to 34)
Bacterial			
Septicemia	1, 2, 2‡, 3, 4, 4, 7, 9, 12§‖, 12§, 15, 15	19‖, 20	34
Pneumonia (nonbacteremic)	13‡, 15, 18‡	20, 20, 20, 24, 24	
Other sinopulmonary	3, 6, 13, 13, 15, 15, 18	19, 20, 20, 24	
Cellulitis or abscess	6, 8, 8	23	30
Urinary tract			31
Interstitial pneumonia			
Unknown etiology	4‡, 7‡, 8‡, 10‡, 12, 16, 18‡		31, 33
Pneumocystis carinii	14‡		25‡, 26‡, 30‡
Cytomegalovirus¶	1‡, 3‡, 8‡, 9‡, 15		
Varicella-zoster¶	5‡		33‡
Other viral			
Varicella-zoster	3		34
Herpes simplex	10, 15		25
Hepatitis	17		
Fungal			
Aspergillosis	5‡, 14‡		
Candidiasis	1‡	21‡	

* Numbers correspond to patients as listed in Table I. Multiple listings of the same number indicate multiple separate infections in the same patient. Diagnostic criteria for these infections have been previously discussed (3, 4).
‡ Infection considered to be a major immediate cause of death. Fatal infections noted in patients 4 and 21 were associated with leukemic relapse.
§ Bacteremia associated with pneumonia.
‖ Bacteremia associated with meningitis.
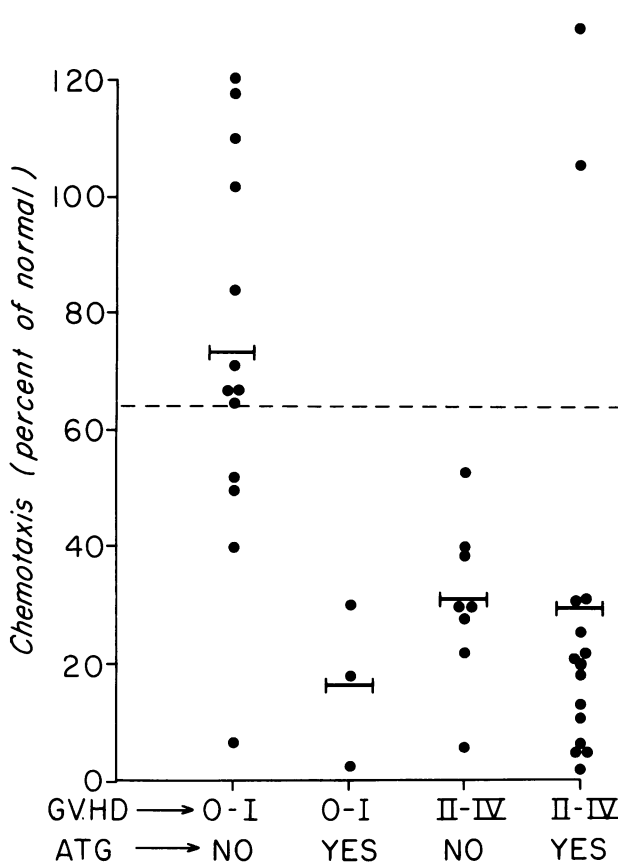¶ Extrapulmonary infection also present in all cases.

FIGURE 1 Neutrophil chemotactic responses as a function of the presence of GVHD and the administration of ATG. Each point represents the average response for a single patient. In the case of patients who changed from one category to another between serial chemotaxis studies, the average response for each appropriate category is depicted. Horizontal bars indicate the means for each category. All values below the dotted line are significantly reduced from normal, while those above are not.

infections and interstitial pneumonia did not correlate with chemotactic responses.

Defective PMN chemotaxis showed striking correlations with GVHD and the recent administration of ATG. Grade II-IV GVHD was present at the time of study in 14 of 18 Group A patients, 3 of 6 Group B patients, and 2 of 10 Group C patients ($P < 0.01$, 3 × 2 chi-square test). Patients having received ATG within 100 days preceding the study (average 22±6 days) were concentrated in Group A (16 of 18 subjects). In contrast, none of 6 in Group B and 2 of 10 in Group C had been so treated ($P < 0.01$, 3 × 2 chi-square test) ; the remaining patients had received either no ATG or in two instances had been treated a long interval before study (> 200 days in patient 20 and > 450 days in patient 29). Severely reduced chemotactic responses therefore occurred

predominantly in patients with Grade II-IV GVHD or recent ATG therapy or both. In fact, all but one patient (number 6) in Group A had one or both of these risk factors present, while 8 of the 10 patients with normal chemotaxis (Group C) had neither of these factors present. Since GVHD and ATG were generally associated, attempts were made to examine them separately. Fig. 1 illustrates the chemotactic responses as a function of GVHD and ATG administration. Some patients appear more than once in Fig. 1 since these individuals changed from one diagnostic category to another between serial chemotaxis studies.

The mean PMN chemotactic response in patients with neither GVHD nor recent administration of ATG was 73.3±9.2%. 9 of these 13 patients were not significantly reduced from normal and only one (patient 6) was severely depressed. This latter patient did, in fact, have mild GVHD (Grade I and thus was placed in the non-GVHD group) when studied, and 18 days later developed progressively severe (Grade III) GVHD. In 15 patients with Grade II-IV GVHD treated with ATG, the mean chemotactic response was 29.7±9.6% ($P < 0.01$ vs. non-GVHD, non-ATG group, t test), and the median was 20%. 13 in this category were in the severely depressed chemotaxis group, and normal responses were seen in the remaining 2. Both GVHD and ATG administration were implicated since the presence of either factor alone was associated with chemotactic responses significantly less than those in patients with neither factor present. Thus, the response in GVHD patients not treated with ATG averaged 31.1±4.9% ($P < 0.01$) in eight subjects. The effect of ATG in the absence of Grade II-IV GVHD was examined in a small group of three patients, two of whom (numbers 2 and 11) received ATG as part of their pregraft preparation, and one (number 16) who was treated for early Grade I GVHD. The mean response in these three patients was 17.0±7.8 ($P < 0.02$).[3]

Serial chemotaxis studies in some patients were of interest in assessing the role of GVHD and ATG. Patient 10 with Grade IV GVHD had responses before and 18 days after treatment with ATG of 31 and 5%, respectively. Patient 13 had a response of 22% in the presence of GVHD ; this decreased to 7% one day after ATG, although subsequent values 30 and 116 days after ATG were 33 and 28%, respectively. On the other hand, patient 17 with GVHD had nearly identical chemotaxis values of 30 and 31% before and 3 days after ATG. In patient 19 (who received no ATG), values of 37 and

---

[3] One identical twin (patient 27) received a syngeneic graft and was thus not at risk for GVHD ; this patient had normal chemotaxis. Since initial submission of the manuscript, three additional twins with syngeneic grafts have been evaluated and found to have normal PMN chemotaxis (95.1, 78.4, and 71.5%).

42% were seen before GVHD, and 39% after developing chronic GVHD. Interestingly, patient 29 was doing well 1½ yr after transplantation and had normal chemotaxis (102%), but after leukemic relapse and a repeat transplant (new patient number 5), developed GVHD, was given ATG, and on two occasions had severely reduced PMN chemotaxis (4 and 8%).

In vitro effect of ATG. Further evidence for a suppressive effect of ATG on chemotaxis was obtained by treating normal PMNs with ATG in vitro. A potent inhibitory effect was observed (see Fig. 2). The highest dilutions showing significant inhibition were 1:4,000, 1:1,000 and 1:100 for the rabbit, horse, and goat preparations, respectively. Normal rabbit serum at dilutions of 1:100 or 1:1,000 had no inhibitory effect. This inhibition by ATG was not attributable to reversal of the chemotactic gradient or to chemotactic deactivation, since ATG was also inhibitory when mixed with the chemotactic factor, and when ATG alone was placed in the lower compartment no chemotactic activity was detected. Using a 1:100 dilution of rabbit ATG the chemotactic inhibitory effect was not reversible by washing and resuspending the PMNs in fresh medium (mean inhibitory activity with and without washing 94.5 and 95.9%, respectively). Thus, the inhibition appeared to be due to a direct effect of ATG on the PMNs rather than an inactivation of the chemotactic attractant. Absorption of rabbit ATG with three serial changes of normal human PMNs resulted in a final supernate which caused 64.0% inhibi-

tion of chemotaxis compared to 96.5% inhibition by control ATG carried through identical processing except for omission of the PMNs ($P < 0.02$). This partial absorption of inhibitory activity by neutrophils is compatible with the presence of antineutrophil antibody in the ATG. The chemotactic inhibitory activity did not appear to result from a cytotoxic effect of ATG on the neutrophils; using the same conditions as those employed in the chemotaxis assay (see Methods), neither rabbit, horse, nor goat ATG in dilutions of 1:100 or 1:1,000 caused any release of ^{51}Cr above negative control values.

Serum chemotactic activity and inhibitors. The PMN chemotactic activity generated by activation of patients' serum with endotoxin was not significantly reduced from normal in all but five subjects (numbers 1, 8, 20, 25, and 30). Activity in these five sera ranged from 13 to 55% of normal. Serum chemotactic activity did not show significant correlation with PMN chemotactic responses or with the clinical parameters examined, although four of the five patients had GVHD and three had received ATG.

Chemotactic inhibitory activity was detected in serum from seven patients (numbers 6, 12, 13, 14, 20, 23, and 28), while serum from all other patients was not significantly inhibitory. Migration was inhibited from 60 to 90% when the cells in the upper compartment of the chemotaxis chamber were suspended in these seven sera as compared to normal control sera. This inhibitory effect was not due to reversal of the chemotactic factor gradient, since nonactivated serum in the lower chamber compartment exhibited a level of chemotactic activity in these seven sera which was equivalent to that of normal control sera. Although 6 of these 7 patients also had reduced chemotactic responses of their PMNs, it appeared that a defective cellular response could not be attributed to a serum inhibitor in the majority of cases since 18 of the 24 patients with depressed PMN chemotaxis did not have detectable inhibitory activity. Of the seven patients with inhibitors only three had received recent ATG. Chronic GVHD was associated with especially potent serum chemotactic inhibitors in three of the four patients with this disorder (numbers 13, 20, and 23).

DISCUSSION

The studies demonstrate defective neutrophil chemotaxis in some patients after bone marrow transplantation. In this group of 34 marrow graft recipients, abnormal chemotaxis was found in 24 instances, and this defect was clearly correlated with the presence of GVHD, the administration of ATG, and a markedly increased incidence of infections. Separate analysis of the effect of GVHD or ATG suggested that each may be acting independently to suppress chemotactic responses. However,
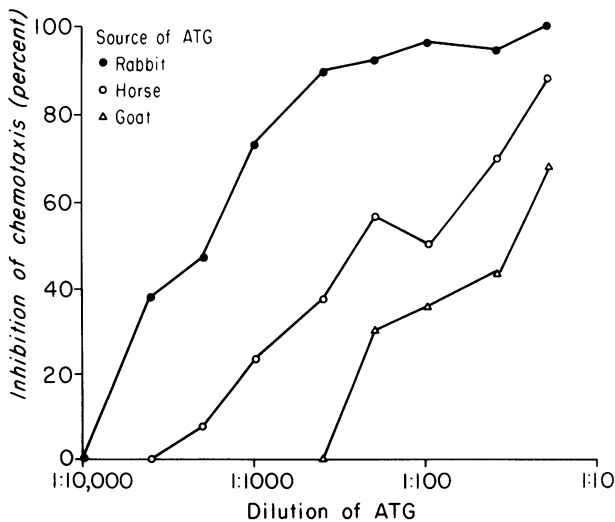

FIGURE 2 In vitro effect of ATG on PMN chemotaxis. The chemotactic response to C5a of normal PMNs suspended in various dilutions of ATG was determined. Data are expressed as percent inhibition relative to control PMN suspensions containing no ATG. Each point represents the mean of three to five separate experiments.

in the majority of instances both factors were present, since the primary use of ATG is in the therapy of GVHD. There was a tendency for better PMN chemotactic responses in patients with acute lymphocytic leukemia as compared to those with aplastic anemia or acute myelocytic leukemia. No correlation was seen with age, sex, time since transplantation, granulocyte counts, or drug therapy. It should be noted that marrow transplant patients tend to have extremely complex clinical courses with multiple interrelated problems and it is often difficult to determine which factors are primary and which are secondary.

A markedly increased incidence of infections has been previously noted in patients with GVHD. In 61 marrow transplant patients from this institution, fatal infections were observed in 26 of 36 with severe GVHD, but in only 4 of 25 with minimal or no GVHD (2). The known depression of immunologic reactivity (5–8) was proposed as an explanation for this observation. The current results suggest that dysfunction of phagocytic cells such as neutrophils may be important as well. The basis for impaired neutrophil chemotaxis in GVHD remains unknown.

Because of the high incidence of fatal GVHD in marrow graft recipients the use of ATG has recently been advocated for the treatment of established GVHD (14). Although this has favorably modified the graft vs. host reaction, many treated patients develop fatal infections, particularly viral interstitial pneumonitis (14). Administration of ATG to renal transplant patients has been associated with an increased incidence of infections due primarily to bacterial pathogens (23). Lymphocytes from ATG treated patients do not react to allogeneic cells in mixed leukocyte cultures due in part to a plasma inhibitor which may persist for weeks after discontinuing ATG (14). One of the toxic effects observed with ATG was the development of myelosuppression with granulocytopenia in a small proportion of patients (14). A slight decrease in neutrophil bactericidal capacity has been reported in ATG treated kidney allograft recipients (24). The current study raises the possibility that ATG treatment may have deleterious effects on neutrophil chemotaxis.

The mechanism of interference with PMN chemotaxis by ATG has not been determined. However, the in vitro demonstration of a direct suppressive effect of ATG on chemotaxis of normal PMNs suggests that this material could have a similar effect in vivo. Interestingly, ATG employed under conditions which markedly suppressed PMN chemotaxis had no detectable cytotoxic effect on PMNs as judged by a ^{51}Cr-release assay. ATG is a globulin fraction from the serum of animals immunized with human thymus (14) and may contain antibodies with specificity or cross-reacting activity against cells other than thymocytes or lymphocytes, e.g. neutrophils. If this were the case, the potential for PMN dysfunction might be abrogated by absorption with neutrophils. Indeed, significant reduction in chemotactic inhibitory activity was achieved by absorbing ATG with normal PMNs; however, the partial nature of this reduction under the conditions employed suggests the presence of both absorbable and nonabsorbable inhibitors. Absorption with erythrocytes has been proposed to avoid the hemolytic anemia seen in some ATG treated patients (14). Unfortunately, absorption might also reduce the immunosuppressive properties of ATG.

Factors in addition to GVHD and ATG administration could well be involved in the diminished chemotactic response. Although no other specific factors were identified, the finding of depressed chemotaxis in a limited number of patients without GVHD or ATG administration supports such a possibility. Furthermore, the presence of both GVHD and ATG treatment was not invariably associated with defective chemotaxis since 2 of 15 such patients had normal migration.

In most patients a cellular abnormality was implicated as the basis for defective PMN chemotaxis. In some subjects the neutrophils may have been damaged *in situ* by a humoral agent such as an antineutrophil component of ATG. However, serum inhibitors of chemotaxis were detected in only 6 of 24 patients with diminished PMN migration and in 1 with a normal response. Hyperimmunoglobulinemia E was noted primarily in patients with GVHD. The role of this abnormality in the chemotactic defect remains unknown, although the levels observed were not as markedly elevated as in most of the patients described with high IgE and depressed PMN chemotaxis (18–22). Serum chemotactic activity was decreased in 5 of 34 patients. A similar serum defect has been described in a single marrow transplant patient (8); the decreased serum opsonic activity also observed in this patient appeared to be related to a serum inhibitor of opsonization.

Numerous examples of an increased incidence of infections in patients with chemotactic abnormalities have been described (for reviews see 25–27). The patients in the current series with decreased PMN chemotaxis had a marked and statistically significant increase in incidence of infections relative to those with normal PMN responses. Bacterial infections including septicemia, meningitis, cellulitis, abscesses, pneumonitis, and other sinopulmonary infections were primarily responsible. In contrast, viral infections and interstitial pneumonia occurred with equal frequency in patients with decreased or normal chemotaxis. The data suggest that neutrophil dysfunction may be at least partially responsible for the high incidence of bacterial and possibly fungal infections in bone marrow transplant patients, particularly those

with GVHD. It has been shown that infection itself may in some instances depress the PMN chemotactic response (28–31). This effect may be of particular significance in severely ill patients with toxic neutrophils (28), a finding not encountered at the time of study in our patients. Several other reports have noted a consistent enhancement of PMN chemotaxis in most patients with acute pyogenic infections (32–34). The latter studies dealt primarily with children and young adults, an age range similar to that of the marrow transplant patients evaluated in the current report.

The findings reported here may have implications for the management of bone marrow transplant patients. Even after engraftment with the appearance of normal numbers of blood granulocytes, serious and recurrent infections remain a major risk. Suspected infection must be treated vigorously, especially in patients who have manifestations of GVHD or who have been treated with ATG. Although the use of ATG has proven to be valuable in suppressing the graft vs. host response (14), the high incidence of infections in ATG-treated patients (14, 23), together with the current results demonstrating suppression of PMN chemotaxis, suggest the need for continued evaluation of improved means for preparing and administering this agent. Finally, the use of granulocyte transfusions in the treatment of infections should be considered not only in granulocytopenic patients, but also in those who may have neutrophil dysfunction.

ACKNOWLEDGMENTS

REFERENCES

1. Thomas, E. D., R. Storb, R. A. Clift, A. Fefer, F. L. Johnson, P. E. Neiman, K. G. Lerner, H. Glucksberg, and C. D. Buckner. 1975. Bone-marrow transplantation. *N. Engl. J. Med.* 292: 832–843, 895–902.
2. Glucksberg, H., R. Storb, A. Fefer, C. D. Buckner, P. E. Neiman, R. A. Clift, K. G. Lerner, and E. D. Thomas. 1974. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation (Baltimore).* 18: 295–304.
3. Neiman, P., P. B. Wasserman, B. B. Wentworth, G. F. Kao, K. G. Lerner, R. Storb, C. D. Buckner, R. A. Clift, A. Fefer, L. Fass, H. Glucksberg, and E. D. Thomas. 1973. Interstitial pneumonia and cytomegalovirus infection as complications of human marrow transplantation. *Transplantation (Baltimore).* 15: 478–485.
4. Clift, R. A., C. D. Buckner, A. Fefer, K. G. Lerner, P. E. Neiman, R. Storb, M. Murphy, and E. D. Thomas. 1974. Infectious complications of marrow transplantation. *Transplant. Proc.* 6: 389–393.

5. Halterman, R. H., R. G. Graw, Jr., D. A. Fuccillo, and B. G. Leventhal. 1972. Immunocompetence following allogeneic bone marrow transplantation in man. *Transplantation (Baltimore).* 14: 689–697.
6. Fass, L., H. D. Ochs, E. D. Thomas, E. Mickelson, R. Storb, and A. Fefer. 1973. Studies of immunological reactivity following syngeneic or allogeneic marrow grafts in man. *Transplantation (Baltimore).* 16: 630–640.
7. Ochs, H. D., R. Storb, E. D. Thomas, H-J. Kolb, T. C. Graham, E. Mickelson, M. Parr, and R. H. Rudolph. 1974. Immunologic reactivity in canine marrow graft recipients. *J. Immunol.* 113: 1039–1057.
8. Bleyer, W. A., R. M. Blaese, J. S. Bujak, G. P. Herzig, and R. G. Graw, Jr. 1975. Long term remission from acute myelogenous leukemia after bone marrow transplantation and recovery from acute graft-versus-host reaction and prolonged immunoincompetence. *Blood.* 45: 171–181.
9. Thomas, E. D., and R. Storb. 1970. Technique for human marrow grafting. *Blood.* 36: 507–515.
10. Thomas, E. D., C. D. Buckner, R. A. Clift, L. Fass, A. Fefer, K. G. Lerner, P. Neiman, N. Rowley, and R. Storb. 1973. Marrow grafting in patients with acute leukemia. *Transplant. Proc.* 5: 917–922.
11. Storb, R., E. D. Thomas, C. D. Buckner, R. A. Clift, F. L. Johnson, A. Fefer, H. Glucksberg, E. R. Giblett, K. G. Lerner, and P. Neiman. 1974. Allogeneic marrow grafting for treatment of aplastic anemia. *Blood.* 43: 157–180.
12. Storb, R., G. L. Floersheim, P. L. Weiden, T. C. Graham, H-J. Kolb, K. G. Lerner, M-L. Schroeder, and E. D. Thomas. 1974. Effect of prior blood transfusions on marrow grafts: abrogation of sensitization by procarbazine and antithymocyte serum. *J. Immunol.* 112: 1508–1516.
13. Lerner, K. G., G. F. Kao, R. Storb, C. D. Buckner, R. A. Clift, and E. D. Thomas. 1974. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HL-A-matched sibling donors. *Transplant. Proc.* 6: 367–371.
14. Storb, R., E. Gluckman, E. D. Thomas, C. D. Buckner, R. A. Clift, A. Fefer, H. Glucksberg, T. C. Graham, F. L. Johnson, K. G. Lerner, P. E. Neiman, and H. Ochs. 1974. Treatment of established human graft-versus-host disease by antithymocyte globulin. *Blood.* 44: 57–75.
15. Gallin, J. I., R. A. Clark, and H. R. Kimball. 1973. Granulocyte chemotaxis: an improved in vitro assay employing chromium-51 labeled granulocytes. *J. Immunol.* 110: 233–240.
16. Gallin, J. I., R. A. Clark, and M. M. Frank. 1975. Kinetic analysis of chemotactic factor generation in human serum via activation of the classical and alternate complement pathways. *Clin. Immunol. Immunopathol.* 3: 334–346.
17. Clark, R. A., and H. R. Kimball. 1971. Defective granulocyte chemotaxis in the Chediak-Higashi syndrome. *J. Clin. Invest.* 50: 2645–2652.
18. Clark, R. A., R. K. Root, H. R. Kimball, and C. H. Kirkpatrick. 1973. Defective neutrophil chemotaxis and cellular immunity in a child with recurrent infections. *Ann. Intern. Med.* 78: 515–519.
19. Hill, H. R., and P. G. Quie. 1974. Raised serum IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. *Lancet.* I: 183–187.

20. Hill, H. R., H. D. Ochs, P. G. Quie, R. A. Clark, H. F. Pabst, S. J. Klebanoff, and R. J. Wedgwood. 1974. Defect in neutrophil chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. *Lancet.* **II**: 617–619.

21. Van Scoy, R. E., H. R. Hill, R. E. Ritts, Jr., and P. G. Quie. 1975. Familial neutrophil chemotaxis defect, recurrent bacterial infections, mucocutaneous candidiasis and hyperimmunoglobulinemia E. *Ann. Intern. Med.* **82**: 766–771.

22. Pincus, S. H., I. T. Thomas, R. A. Clark, and H. D. Ochs. 1975. Defective neutrophil chemotaxis with variant icthyosis, hyperimmunoglobulinemia E and recurrent infections. *J. Pediatr.* **87**: 908–911.

23. Michel, R. P., R. D. Guttmann, J. Knaack, J. Klassen, J.-G. Beaudoin, and D. D. Morehouse. 1975. Antilymphocyte globulin in renal transplantation. Nephrotic syndrome and infection as possible complications. *Arch. Surg.* **110**: 90–93.

24. Grogan, J. B., and G. V. Smith. 1975. Neutrophil function in clinical kidney allograft recipients. *Surgery (St. Louis).* **78**: 316–321.

25. Ward, P. A. 1974. Leukotaxis and leukotactic disorders. *Am. J. Pathol.* **77**: 520–538.

26. Miller, M. E. 1975. Pathology of chemotaxis and random mobility. *Semin. Hematol.* **12**: 59–82.

27. Snyderman, R., M. C. Pike, and L. C. Altman. 1975. Abnormalities of leukocyte chemotaxis. *Ann. N. Y. Acad. Sci.* **256**: 386–401.

28. McCall, C. E., J. Caves, R. Cooper, and L. DeChatelet. 1971. Functional characteristics of human toxic neutrophils. *J. Infect. Dis.* **124**: 68–75.

29. Mowat, A. G., and J. Baum. 1971. Polymorphonuclear leukocyte chemotaxis in patients with bacterial infections. *Br. Med. J.* **3**: 617–619.

30. Baisero, M. H. 1973. Chimiotactisime des polynucléaires humains in vitro. III. Etude de l'infection aiguë et chronique de l'adulte. *Schweiz. Med. Wochenschr.* **103**: 1599–1605.

31. Anderson, R., R. Sher, A. R. Rabson, and H. J. Koornhof. 1974. Defective chemotaxis in measles patients. *S. Afr. Med. J.* **48**: 1819–1820.

32. Hill, H. R., J. M. Gerrard, N. A. Hogan, and P. G. Quie. 1974. Hyperactivity of neutrophil leukotactic responses during active bacterial infection. *J. Clin. Invest.* **53**: 996–1002.

33. Hill, H. R., E. L. Kaplan, A. S. Dajani, L. W. Wannamaker, and P. G. Quie. 1974. Leukotactic activity and reduction of nitroblue tetrazolium by neutrophil granulocytes from patients with streptococcal skin infection. *J. Infect. Dis.* **129**: 322–326.

34. Hill, H. R., W. J. Warwick, J. Dettloff, and P. G. Quie. 1974. Neutrophil granulocyte function in patients with pulmonary infection. *J. Pediatr.* **84**: 55–58.