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Effect of Viral and Bacterial Pneumonias on Cell-Mediated Immunity in Humans

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Cell-mediated immunity (CMI) was assessed during infection and after convalescence in 12 patients with influenza pneumonia and 10 patients with bacterial pneumonia. The patients with influenza pneumonia had a marked impairment of skin test reactivity, and their lymphocytes showed a diminished response to phytohemagglutinin and streptokinase-streptodornase stimulation in vitro. Suppression of CMI was related to the severity of the pneumonia. Patients with bacterial pneumonia showed as great a suppression of the response to phytohemagglutinin and streptokinase-streptodornase as the patients with viral pneumonia. All parameters of CMI returned to normal in both groups after convalescence. The depression of CMI could not be related to a decrease in the number of thymus-derived lymphocytes or to serum-suppressive factors in these patients.

Cell-mediated immunity (CMI) in humans, assessed by skin testing or in vitro lymphocyte transformation, has been shown to be depressed during various viral illnesses (15, 21, 27) and after vaccination for a number of viral diseases (7, 17). We have shown previously that infections due to influenza A or rhinoviruses, localized to the upper respiratory tract, do not cause a suppression of systemic CMI (14). Influenza pneumonia with spread of the virus throughout the lungs is a more serious illness and more likely to lead to viremia than mild upper respiratory tract influenza (18, 20). This study was done to assess the influence of this more severe type of influenza infection on systemic CMI. As a control group, patients with bacterial pneumonia were studied. There is a paucity of data regarding the effect of acute bacterial infections on CMI (1, 19, 24) and no previous work investigating CMI during bacterial pneumonias. We found that both viral and bacterial pneumonias in humans are associated with significant suppression of CMI.

MATERIALS AND METHODS

Patient population. Twenty-two patients with pneumonia treated at the Cincinnati General Hospital and the Cincinnati Veterans Administration Hospital were included in this study. Twelve patients had influenza pneumonia. The average age was 53 years with a range from 32 to 81 years. Infection with influenza A/England/42/72 in nine patients and influenza A/Port Chalmers/1/73 in three patients was demonstrated by isolation of the virus from the nasopharynx or sputum (seven patients) and/or a fourfold change in the titer of complement-fixing antibody to influenza A (twelve patients). Antibody titers were measured in a microtiter system described by Sever (28). All patients had pulmonary infiltrates on chest roentgenograms. Three patients had documented pulmonary superinfection with *Staphylococcus aureus* or *Streptococcus pneumoniae*. In two patients, superinfection occurred after the initial studies of lymphocyte function were completed. One patient had both pneumococcal and influenza pneumonia at the time of study. No patient received amantadine, corticosteroids, or aspirin. Those with documented bacterial infection received penicillin. Six patients were judged to be severely ill with marked hypoxemia ($pO_2 < 30$ mm Hg) and diffuse alveolar infiltrates. All required endotracheal intubation and mechanical ventilation; four of these patients died. Six patients had only moderate respiratory distress and less extensive pulmonary infiltrates. The patients did not require mechanical ventilation or intubation, and they all survived.

All patients with influenza were studied within 36 h of admission to the hospital; follow-up studies in survivors were accomplished in two patients 20 days after the onset of illness and in three patients 46 to 50 days after their illness began.

Ten patients with bacterial pneumonias were studied. The average age of these patients was 54 years with a range of 28 to 75 years. All had purulent sputum and radiographic evidence of pulmonary infiltrates. Viral cultures were negative, and antibody titers to influenza did not change in two patients in whom these parameters were studied. *S. pneumoniae* was isolated from the sputum of five patients, whereas typical gram-positive, lancet-shaped diplococci were seen in the sputum smears from two other patients. Sputum from three patients grew only *Haemophilus influenzae* and had small,

gram-negative coccobacilli in the smear. Treatment in seven patients consisted of penicillin and in three patients, ampicillin. Studies were done on these patients during the first 3 days of hospitalization; follow-up studies were done 1 month later.

The concomitantly studied control population consisted of 26 healthy persons 21 to 41 years of age.

Skin testing. Intradermal skin testing was performed by one observer with 0.1 ml of the following antigens: purified protein derivative, 5 tuberculin units (Parke-Davis, Inc., Detroit, Mich.); histoplasmin (Parke-Davis, Inc.); mumps virus (Eli Lilly, Inc., Indianapolis, Ind.); streptokinase-streptodornase (SK-SD), 7 U of SK and 2.5 U of SD (Lederle, Inc., Pearl River, N.Y.). Induration greater than 5 mm at 48 h was judged to be positive.

Lymphocyte cultures. Lymphocyte cultures were prepared from peripheral blood as described previously, with 2.5×10^5 mononuclear cells suspended in Eagle minimum essential medium with 20% heat-inactivated fetal calf serum in a total volume of 0.2 ml (15). Phytohemagglutinin-M (PHA) (Difco, Inc., Detroit, Mich.) was reconstituted in 5 ml of phosphate-buffered saline, pH 7.3. Amounts varying from 1 to 30 μl were added to triplicate sets of cultures. Preservative-free SK-SD (kindly supplied by Lederle Laboratories, Pearl River, N.Y.), reconstituted in phosphate-buffered saline, was added to triplicate sets of cultures in amounts varying from 50 to 150 U of SK (12.5 to 37.5 U of SD). Control cultures contained no mitogen or antigen.

The effect of the patient's plasma on the ability of his lymphocytes to respond to PHA was investigated by substituting 20% heat-inactivated autologous plasma for the fetal calf serum in the cultures.

All lymphocyte cultures were harvested and prepared for beta liquid scintillation counting as previously outlined (15). Lymphocyte deoxyribonucleic acid (DNA) synthesis was expressed as disintegrations per minute per culture.

Thymus-derived lymphocytes. Thymus-derived (T) lymphocytes were enumerated by the formation of spontaneous rosettes with sheep erythrocytes according to the method of Jondal et al. (11). Lymphocytes with three or more sheep erythrocytes attached were scored as T cell rosettes.

C-reactive protein and α_2-macroglobulins. The presence of C-reactive protein in the serum was determined by the agglutination of latex particles coated with antiserum against C-reactive protein (Sylvana, Inc., Millburn, N.J.). Serum α_2-macroglobulin levels were quantitated by radial immunodiffusion with goat antiserum against human α_2-macroglobulin (Hyland Laboratories, Costa Mesa, Calif.).

Statistical methods. Data were analyzed by the Wilcoxon rank sum test, chi-square analysis with Yates correction, and correlation coefficients.

RESULTS

Skin tests. Delayed hypersensitivity to four skin test antigens was assessed in nine persons with influenza pneumonia (Table 1). Only 6 of 35 skin tests performed during the illness were positive. Six persons, five of whom had severe illness, were anergic to all antigens. Follow-up studies showed that 13 of 24 skin tests were positive. Tests could not be repeated on three anergic patients who died.

Response to PHA and SK-SD during influenza pneumonia. The patients with influenza pneumonia showed a depression of the expected response to PHA (Fig. 1). The mean response of the patients with influenza (5,977 dpm) was significantly less than that shown by the control subjects (20,930 dpm) ($P < 0.001$ by the Wilcoxon rank sum test). The response was within the normal range in five patients and markedly low in seven. Six of those seven patients were skin tested and found to be anergic during the illness. A poor response to PHA was significantly correlated with the severity of the patient's illness ($P < 0.02$ by chi-square test). All patients with severe disease responded very poorly to PHA, whereas the response in pa-

TABLE 1. *Delayed hypersensitivity to four skin test antigens in nine patients with influenza pneumonia*

Patient	Skin test reactivity (mm of induration)							
	During influenza				After convalescence			
	Mumps	Histoplasmin	SK-SD	PPD	Mumps	Histoplasmin	SK-SD	PPD
1[a]	0	0	0	0	ND[b]	ND	ND	ND
2[a]	0	0	0	0	ND	ND	ND	ND
3[a]	0	0	0	0	ND	ND	ND	ND
4	0	0	0	0	5	12	0	0
5	0	0	0	0	5	20	10	0
6	3	0	0	0	12	18	0	5
7	ND	15	4	0	0	0	10	0
8	0	10	14	14	0	20	10	10
9	10	0	30	0	10	0	60	0

[a] Patient died during illness.
[b] ND, Not determined.

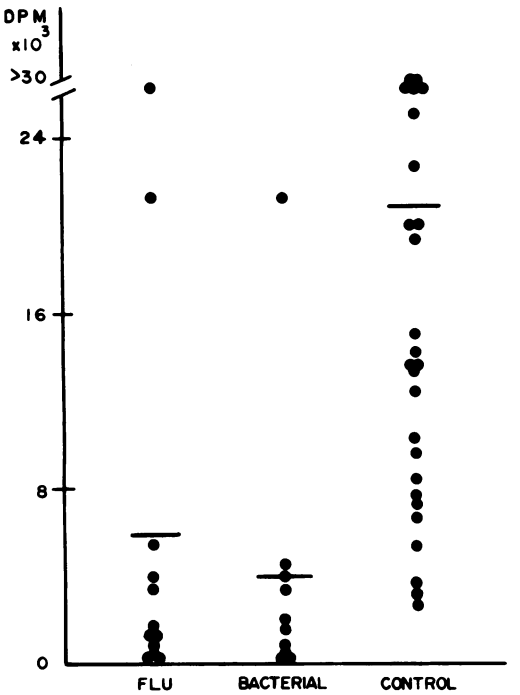

FIG. 1. *Maximum response to PHA, expressed as disintegrations per minute per culture (DPM), for subjects with influenza (FLU) and bacterial pneumonias and control subjects. Mean values for each group are shown by the horizontal bar.*

tients with moderate disease was variable. There was no correlation shown between the responses to PHA and the total leukocyte count ($r = -0.42$) or the total number of neutrophils ($r = -0.46$). No correlation could be found between the titer of antibody to influenza A and the response to PHA ($r = -0.24$). C-reactive protein was found in four patients, only one of whom showed poor PHA stimulation in vitro. No patient with influenza had an elevation in the serum α_2-macroglobulin fraction.

Increased spontaneous transformation was not observed in cultures to which no mitogen had been added. Dose-response curves to three different doses of PHA were performed in three patients and showed the same shape as those of control subjects, although the response to each dose of PHA was lower for the patients with influenza than the controls.

Five patients were studied after convalescence from influenza. (Four patients with severe illness died, and three patients were lost to follow-up.) The five patients retested showed a good response to PHA and did not differ from the controls in this response. (Mean dpm for the five patients was 19,068; mean dpm for the controls, 20,930.)

During the acute stages of the illness only 2 of 11 patients tested showed lymphocyte stimulation by SK-SD (Fig. 2). These same two patients also showed the greatest PHA stimulation. The poorest response to SK-SD was shown by those patients who were unresponsive to PHA. Follow-up testing with SK-SD in vitro was done in only four patients, three of whom did not respond to SK-SD during the illness. (Four other patients died and three were lost to follow-up.) Three of the four tested were skin test positive to SK-SD and responded to SK-SD in vitro. One patient remained skin test negative and did not show enhanced DNA synthesis in vitro to SK-SD even after convalescence.

Response to PHA and SK-SD during bacterial pneumonia. Patients with bacterial pneumonia showed a response to PHA not unlike that shown by the patients with influenza (Fig. 1). The mean response (3,822 dpm) was significantly less than that of the control subjects ($P < 0.001$ by the Wilcoxon rank sum test), but not significantly different from the patients with influenza pneumonia ($P > 0.1$). Six patients showed a markedly abnormal response, whereas values for four others fell in the normal range. Cultures that contained no mitogen

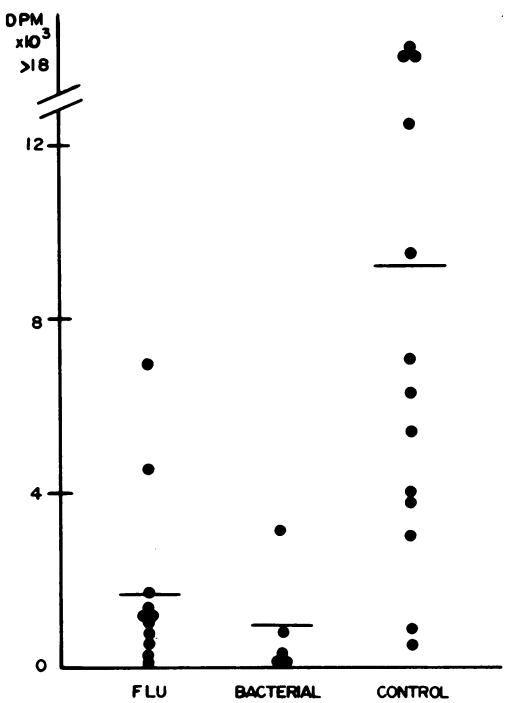

FIG. 2. *Maximum response to SK-SD, expressed as disintegrations per minute per culture (DPM), for subjects with influenza (FLU) and bacterial pneumonias and control subjects. Mean values for each group are shown by the horizontal bar.*

did not show enhanced DNA synthesis. The shape of the dose-response curves to PHA was the same for the patients as the controls. No correlation was found between the response to PHA and leukocytosis ($r = -0.33$) or between the response to PHA and the degree of neutrophilia ($r = -0.34$). The PHA response was not correlated with the extent of the pneumonic process as determined by the number of lobes involved on radiographic examination ($r = -0.18$). C-reactive protein was found in only one patient, and α_2-macroglobulin levels were normal in all patients.

Follow-up studies were accomplished in 5 of the 10 patients. All responded well to PHA after clearing of the pneumonia.

The mean response to SK-SD in patients with bacterial pneumonia was far below that seen in the control subjects (Fig. 2). Only one of five patients showed stimulation by this specific antigen. Follow-up studies with SK-SD were not accomplished in this group.

Effect of autologous plasma on PHA response of patients' lymphocytes. Substitution of the fetal calf serum by autologous plasma in cultures of lymphocytes from patients with either viral or bacterial pneumonia did not change the response of the lymphocytes to PHA (Table 2). Lymphocytes from only three patients with influenza (those with infection due to influenza A/Port Chalmers/1/73) were studied in both autologous plasma and fetal calf serum. No suppressive effect of autologous plasma was noted, and, in fact, two patients responded better with their own plasma than with the fetal calf serum. The mean response to PHA of seven patients with bacterial pneumonia was not significantly different in autologous plasma when compared with fetal calf serum.

TABLE 2. *Comparison of the PHA response in autologous plasma and fetal calf serum*

Patient	Pneumonia	Lymphocyte DNA synthesis (dpm) (PHA-treated cultures)	
		Autologous plasma	Fetal calf serum
1	Influenza	7,983	1,367
2	Influenza	23,060	31,377
3	Influenza	18,425	3,457
4	Bacterial	2,646	3,982
5	Bacterial	1,971	1,573
6	Bacterial	804	454
7	Bacterial	5,186	4,659
8	Bacterial	7,514	2,028
9	Bacterial	422	244
10	Bacterial	2,310	338
Controls (9)		16,906[a] (2,322–38,044)	28,355 (3,368–81,474)

[a] Mean with range.

Thymus-derived lymphocytes. T lymphocytes were measured during illness in three patients with influenza pneumonia and four with pneumococcal pneumonia (Table 3). One patient had a low percentage of T cells (52% in a patient with influenza), but no patient showed a decrease in the total number of circulating T cells.

DISCUSSION

These studies reveal that patients with viral and bacterial pneumonia manifest a transient depression of systemic CMI during the acute illness. This suppression is shown in the delayed hypersensitivity response to skin test antigens as well as the in vitro stimulation of lymphocytes by PHA and SK-SD.

Our preceding study revealed that both naturally acquired influenza A/Eng/42/72 infection and experimentally induced influenza A/Hong Kong/8/68 infection localized to the upper respiratory tract were not associated with a suppression of CMI (14). In contrast, the patients in this study, all of whom had more severe influenza infection associated with pulmonary infiltrates, showed a significant suppression of both in vivo and in vitro parameters of CMI. Within this group of patients with pneumonia, those who had the most severe infection (as defined by marked hypoxemia and the need for mechanical ventilation) were anergic and unresponsive to both PHA and SK-SD. Those who had less severe illness showed a variable amount of immunosuppression, but none were both anergic to skin testing and unresponsive to PHA and SK-SD in vitro. Our studies thus imply that infection with the influenza virus does not necessarily always lead to immunosuppression, but that this immunosuppression seems to be related to the severity of the infection.

Varying results have been obtained in previous studies of CMI during influenza (2, 3, 13, 14, 25, 26). Other authors have not emphasized

TABLE 3. *Thymus-derived lymphocytes in patients with pneumonia*

Subjects	No.	T cells	
		%	No. 9/mm³
Influenza pneumonia	3	62.7[a] (52–71)	1145 (946–1,401)
Bacterial pneumonia	4	70.5 (63–79)	1,476 (616–2,972)
Controls	24	67.2 ± 6.2[b]	1,658 ± 500

[a] Mean with range.
[b] Mean ±1 standard deviation.

the relationship to severity of the illness, and, indeed, Kantzler et al. (13) found a decrease in skin test reactivity and PHA responsiveness after mild influenza. Reed et al. (26) did not differentiate between their patients with upper respiratory tract symptoms and those with pneumonia. The response to PHA was normal in their patients. Buckley et al. (3) studied 7 patients with influenza pneumonia caused by influenza A/Hong Kong/8/68 and 11 patients with influenza complicated by bacterial pneumonia. Although the patients of Buckley et al. were similar to those in this study, their technique involved use of a prolonged tritium pulse as compared with our short pulse with tritium. They found a striking increase in background DNA synthesis in lymphocyte cultures obtained from patients with pneumonia. None of the patients in our study showed this phenomenon, but these differences may well relate to the different techniques used for assessing DNA synthesis.

It is possible that, during severe infection, viremia occurred and a direct toxic effect of the virus on the lymphocytes followed. Viremia during the course of influenza is uncommon. The few documented cases manifested viremia during the incubation period when symptoms were absent (16, 29) or in the course of severe influenza (18, 20). Unfortunately, viral cultures of whole-blood or buffy-coat leukocytes were not performed in our patients, so a direct toxic effect is only conjectural.

Factors other than the virus itself may have contributed to the immunosuppression seen in these patients. The results obtained in patients with bacterial pneumonia support this idea. These patients showed as poor a response to both PHA and SK-SD as the patients with viral infection. Factors that could lead to immunosuppression in both bacterial and viral infections include serum proteins such as C-reactive protein and α_2-macroglobulins, both of which have been associated with in vivo and in vitro immunosuppression (4, 5, 8, 10, 22). However, serum levels of neither factor correlated with the depression of CMI found in our patients. It is possible that levels of immunoregulatory α-globulin (IRA) could have been increased, and measurement of total serum α_2-macroglobulins would not reflect this increase.

Leukocytosis is another factor that could relate to immunosuppression during both viral and bacterial infections (9, 23). However, we found no association between immunosuppression and total leukocyte or neutrophil count in either of our patient groups.

A depression in the total number or relative percentage of circulating T cells has been associated with immunosuppression during various infectious diseases (6, 30). This mechanism did not appear to be operative in our patients with either viral or bacterial disease; no decrease in total numbers of T cells was found. It is possible that specific subpopulations of T lymphocytes were influenced by the pulmonary infections and that this effect was not reflected in the total number of rosette-forming cells. It has been suggested that viral infections may lead to enhanced activity of subpopulations such as suppressor T cells with subsequent transient immunosuppression (12).

Finally, it is possible that the immunosuppression found during influenza was indeed mediated directly by the virus and that entirely different mechanisms were operative in patients with bacterial pneumonia.

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