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Immunosuppression During Influenza Virus Infection

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The effects of a live attenuated influenza vaccine and subsequent challenge with virulent influenza virus on the delayed hypersensitivity skin test, and the in vitro response of lymphocytes were evaluated. Volunteers were skin tested before and after administration of vaccine or placebo and challenge with PPD (a purified protein derivative of *Mycobacterium tuberculosis*), candida, mumps, and trichophytin, and their lymphocytes were tested for [^{3}H]thymidine uptake in response to phytohemagglutin. Of eight volunteers who showed evidence of viral replication after administration of the attenuated vaccine, four had a significant diminution in their skin test response, whereas 8 of 13 volunteers infected with virulent influenza virus showed a diminution. Of the 21 volunteers who were infected with either attenuated or virulent influenza virus, 12 showed suppression of their phytohemagglutin response. None of the volunteers who were given placebo vaccine, or who showed no evidence for viral replication after immunization or challenge, had a suppression of their skin test or phytohemagglutin responses. Although most of the infected volunteers demonstrated suppression of their T-cell function, there was no evidence of a similar suppression of B-cell function.

The immunosuppression found in various clinical states is associated with an increased incidence of malignancies (13, 19). The possibility that viruses could suppress their host's immune system was first raised by von Pirquet in 1908 (24); if common viral infections are shown to induce an immunosuppressed state for a significant period of time, an important factor in carcinogenesis might be elucidated. This is especially significant with respect to respiratory viral infections like influenza, rhinovirus, etc., since they are the most common infectious diseases of humans.

In 1919 Bloomfield and Mateer (5) reported 19 consecutive cases of acute influenza in which only one patient had a positive tuberculin reaction, whereas 17 patients had positive reactions 1 to 11 days after the febrile stage subsided. Their observations have recently been confirmed by Reed et al. (27) who found suppression of cutaneous reactions to a purified protein derivative of *Mycobacterium tuberulosis* (PPD), candida, trichophytin, and coccidioidin in seven cases of influenza serologically confirmed. Immunization of normal individuals with killed influenza vaccine had no effect on cutaneous reactivity. Four patients' lymphocytes were studied in vitro at the time of cutaneous anergy and had normal responses to stimulation with phytohemagglutin (PHA). However, another study found significant suppression of PHA stimulation in all its 15 patients with acute influenza (26).

This study was undertaken in volunteers to evaluate the effects of live attenuated influenza vaccine and subsequent challenge with virulent influenza virus (i) on cutaneous reactivity to PPD, candida, mumps, and trichophytin, and (ii) on the in vitro response of lymphocytes.

MATERIALS AND METHODS

Volunteers. Forty normal adult volunteers of both sexes, aged 18 to 35, with neutralizing antibody titers of $<1:8$ to $A_2(H_3N_2)$/Hong Kong influenza virus were selected. A complete medical history and physical examination were done to eliminate volunteers with an underlying medical illness.

Immunization. In a double-blind fashion, volunteers were inoculated intranasally with 10^4 50% tissue culture infectious doses per 0.5 ml of a horse serum-resistant strain of $A(H_3N_2)$/England/8/68 influenza virus, obtained from Smith, Kline, and French Laboratories, or saline placebo. Two months later, all volunteers were challenged with 10^5 50% tissue culture infectious doses of a virulent strain of $A(H_3N_2)$/Hong Kong influenza virus (supplied by Y. Togo, Department of Medicine, University of Maryland School of Medicine).

Evaluation for viral infection. Volunteers were

monitored for virus shedding by twice-daily throat gargles, inoculated onto rhesus monkey kidney tissue culture (18), for serum (8) and nasal secretion antibody rise by the virus neutralization test (32), and for clinical evidence of respiratory infection (11).

Immunological evaluation. Two days prior to virus challenge, blood was drawn for lymphocyte studies, and baseline skin tests to candida, mumps, PPD, and trichophytin were performed. These were repeated 3 days after challenge and once again two weeks after challenge.

(i) Intradermal skin tests were read 48 h after installation and recorded as millimeters of induration.

(ii) **PHA stimulation.** Peripheral leukocytes were separated from the plasma of heparinized blood samples, centrifuged, and washed once in Hanks balanced salt solution. The cells were resuspended in Eagle medium (containing 20% fetal bovine serum, 100 μg of streptomycin, 100 U of penicillin per milliliter, and 0.3 g of bicarbonate per 100 ml) and adjusted to a final concentration of 10^6 leukocytes per ml. Cultures were prepared in quadruplicate with 2 ml of cell suspension per culture. Phytohemagglutinin-M (Difco; 0.05 ml) was added to each culture as a mitogen, and the cultures were incubated in 5% CO_2 at 37 C for 72 h. Deoxyribonucleic acid synthesis was measured by the incorporation of [^{3}H]thymidine into acid-soluble material. [^{3}H]Thymidine (1 μCi) (specific activity of 1.9 Ci/mmol, 1 μCi per 2 μliters, Schwarz BioResearch) was added to the cultures for the last 24 h of incubation. The cultures were then centrifuged, and the cell pellets were washed twice with cold saline and twice with trichloracetic acid at 4 C. The resulting precipitates were then washed once with methanol, air dried, and solubilized (Nuclear Chicago Solubilizer, 0.25 ml per culture). Fifteen milliliters of scintillation fluid (5 g of 2,5-diphenyloxazole-1-benzene per ml in toluene) was added to the solubilized precipitates, and the solutions were transferred to scintillation vials. The vials were counted in a liquid scintillation counter (Beckman, model LS 250). The technique gave counts consistently within 5 to 10% for replicate cultures. The ratio of uptake of thymidine by the stimulated culture to the uptake of a simultaneously incubated unstimulated culture was calculated as the stimulation ratio. The ratio of the uptake of the unstimulated culture of volunteer's lymphocytes to that of control's unstimulated lymphocytes was calculated as the background ratio.

(iii) Antibody to rubella virus was measured by the hemagglutination-inhibition technique previously described (25).

RESULTS

Of the 27 volunteers, 13 received the attenuated vaccine and 8 showed virus replication, as evidenced by a serum or secretory antibody rise (Table 1). A significant reduction in skin test reactivity was scored if all the preinoculation-positive skin tests showed a 50% or greater reduction in the diameter when tested 72 to 96 h after inoculation. Four volunteers were observed to have such a reduction after the first dose of vaccine or placebo (Table 2); all were in the vaccine group and all evidenced viral replication (one had virus isolated from a throat gargle, and all showed a significant antibody rise).

PHA was used as a nonspecific stimulus of blastogenesis of the subjects' peripheral lymphocytes. This was measured by [^{3}H]thymidine uptake and was considered abnormal if the stimulation ratio was ≤ 10 or if the background ratio was ≥ 2.5. After the first dose of vaccine or placebo, seven volunteers developed a transient abnormality of their PHA response, and all were in the vaccine group and all evidenced viral replication (Table 3). Thus, of the eight vaccinees who showed virus replication, all but one developed an abnormal PHA response.

Following the second dose of vaccine or placebo, two volunteers showed virus replication, as evidenced by a fourfold or greater antibody rise. None had a significant reduction in skin test reactivity or an abnormality of their PHA response.

Four to eight weeks after the second dose of vaccine or placebo, the volunteers were challenged with virulent influenza virus. Thirteen of the 27 showed virus replication, as evidenced by a significant antibody rise, and in addition, seven had clinical influenza and four had virus isolated from a throat gargle (Table 1). Eight volunteers were observed to have a significant reduction in their delayed hypersensitivity skin test response, and all had a fourfold or greater rise in serum and/or secretory influenza virus neutralizing antibody (Table 2). Five developed a transient abnormality of their PHA response, and also, they were all infected (Table 3). Of the 13 volunteers who showed an antibody response, 12 developed an abnormality of one of the two parameters of cell-mediated immunity that were measured.

In the volunteers who had a significant reduction in skin test reactivity, the mean diameter changed from 18 to 3 mm. Seven of the 12 showed a complete disappearance of skin test reactivity. In all but one case, the skin test reactivity had returned by the time the volunteers were retested 2 weeks later. In that one individual, the skin test reactivity returned to normal at some point between 2 and 4 weeks after the infection.

The PHA abnormality was of longer duration than the skin test depression in two cases lasting from 2 to 4 weeks. The usual abnormality noted in the PHA response was a decreased stimulation ratio. The one exception was a case in which there was an increased background

TABLE 1. *Evidence of infection following administration of attenuated influenza vaccine or placebo and subsequent challenge with virulent influenza virus*

Volunteers	Inoculum	After vaccine		After challenge		
		Virus isolation[a]	Antibody response[b]	Illness[c]	Virus isolation[a]	Antibody response[b]
1	Vaccine	+	+	−	−	−
2	Vaccine	−	+	−	−	−
3	Vaccine	+	+	−	−	−
4	Vaccine	−	+	−	−	−
5	Vaccine	−	−	−	−	−
6	Vaccine	+	+	−	−	−
7	Vaccine	−	−	−	−	−
8	Vaccine	−	+	−	−	−
9	Vaccine	−	−	+	−	+
10	Vaccine	−	−	−	−	−
11	Vaccine	−	+	−	−	−
12	Vaccine	−	−	−	−	−
13	Vaccine	−	+	−	−	−
14	Placebo	−	−	+	+	+
15	Placebo	−	−	−	−	+
16	Placebo	−	−	−	−	−
17	Placebo	−	−	+	+	+
18	Placebo	−	−	+	−	+
19	Placebo	−	−	−	−	+
20	Placebo	−	−	−	−	+
21	Placebo	−	−	−	−	+
22	Placebo	−	−	−	−	−
23	Placebo	−	−	+	−	+
24	Placebo	−	−	−	−	+
25	Placebo	−	−	+	+	+
26	Placebo	−	−	+	+	+
27	Placebo	−	−	−	−	+

[a] On one or more occasions; isolations were attempted twice daily for 5 days after vaccine or placebo and challenge.

[b] Fourfold or greater rise in serum and/or nasal secretion neutralizing antibody titer.

[c] Febrile upper respiratory infection.

ratio, indicating a high level of spontaneous thymidine incorporation. In one individual both decreased stimulation and increased background ratios appeared.

A comparison of the depression of the skin test and PHA responses shows that, of the 21 instances when volunteers showed evidence of virus replication by one or more of the criteria used, 12 developed suppression of only one of the two parameters tested, with only six instances of both being abnormal simultaneously.

A comparison of immunosuppression in the volunteers who were infected with the attenuated virus with those infected with the virulent virus, i.e., eight volunteers infected with attenuated virus and 13 with the virulent virus, shows that a depression of skin test of PHA reactivity occurred in 7 out of 8 and 11 out of 13 volunteers, respectively.

Thus, the incidence of immunosuppression was not significantly different in those who were infected with the attenuated as compared to the virulent influenza virus.

To assess B-cell function, antibody to rubella virus, an unrelated antigen, was measured before, during, and after influenza virus infection. In no instance was there a significant (fourfold or greater) change in titer during the period of observation.

DISCUSSION

Von Pirquet observed that children lost their skin reactivity to tuberculin a few days prior to the exanthem of measles, gradually becoming positive 5 to 10 days after the rash subsided (24). He also noted that tuberculosis tended to become active after measles. Suppression of the tuberculin test during measles has subsequently been confirmed (3, 14, 21). More recently, live attenuated measles vaccine has been shown to suppress the tuberculin reaction (6, 20, 30), whereas killed vaccine had no effect (10). Live

TABLE 2. *Delayed hypersensitivity skin response in volunteers before and after influenza virus administration*

Volunteers	Inoculum	After attenuated vaccine					After virulent virus challenge				
		Evidence of infection[a]	Skin test[b]				Evidence of infection[c]	Skin test[b]			
			Mumps	Candida	Tri-chophyton	PPD		Mumps	Candida	Tri-chophyton	PPD
1	Vaccine	+	11/11	12/14	10/11	0/0	–	10/8	8/8	13/20	0/0
2	Vaccine	+	10/0[d]	15/0[d]	0/0	0/0	–	8/10	8/6	0/0	0/0
3	Vaccine	+	11/3[d]	14/6[d]	0/0	0/0	–	13/15	15/18	0/0	0/0
4	Vaccine	+	12/10	0/0	0/0	0/0	–	14/16	0/0	0/0	0/0
5	Vaccine	–	12/10	8/10	0/0	0/0	–	10/8	15/18	0/0	0/0
6	Vaccine	+	18/13	20/19	9/8	0/0	–	17/20	ND[e]	10/12	0/0
7	Vaccine	–	25/29	13/21	0/0	0/0	–	20/25	18/22	0/0	0/0
8	Vaccine	+	20/15	15/17	13/19	0/0	–	ND	16/15	14/17	0/0
9	Vaccine	–	10/6	8/13	0/0	0/0	+	11/13	10/5[d]	0/0	0/0
10	Vaccine	–	0/0	2/2	0/0	10/15	–	0/0	4/6	0/0	12/8
11	Vaccine	+	5/0[d]	10/0[d]	0/0	5/0[d]	–	4/5	11/11	0/0	0/0
12	Vaccine	–	30/ND	18/16	0/0	13/12	–	ND	16/12	0/0	12/14
13	Vaccine	+	8/0[d]	5/0[d]	13/0[d]	0/0	–	9/11	4/6	10/13	0/0
14	Placebo	–	10/10	10/12	0/0	11/11	+	8/0[d]	7/0[d]	0/0	11/0[d]
15	Placebo	–	12/12	0/0	0/0	0/0	+	8/14	0/0	0/0	0/0
16	Placebo	–	0/0	0/0	0/0	15/15	–	0/0	0/0	0/0	22/20
17	Placebo	–	10/11	12/11	0/0	0/0	+	20/3[d]	6/0[d]	0/0	0/0
18	Placebo	–	0/0	13/14	10/11	0/0	+	0/0	14/17	8/13	0/0
19	Placebo	–	6/10	11/17	7/10	0/0	+	9/0[d]	11/3[d]	5/0[d]	0/0
20	Placebo	–	18/14	21/22	0/0	0/0	+	14/14	18/20	0/0	0/0
21	Placebo	–	25/12	30/30	5/5	9/15	+	14/6[d]	34/2[d]	5/0[d]	10/0[d]
22	Placebo	–	9/8	10/12	0/0	0/0	–	8/10	12/15	0/0	0/0
23	Placebo	–	4/4	0/0	5/5	0/0	+	6/0[d]	0/0	9/0[d]	0/0
24	Placebo	–	4/4	31/35	6/6	16/12	+	6/0[d]	32/10[d]	8/0[d]	12/0[d]
25	Placebo	–	7/7	10/9	0/0	21/20	+	6/7	11/7	0/0	18/12
26	Placebo	–	4/4	24/22	0/0	15/2[d]	+	5/0[d]	20/0[d]	0/0	14/0[d]
27	Placebo	–	7/8	12/16	0/0	0/0	+	66/0[d]	18/8[d]	0/0	0/0

[a] Virus isolation and/or significant antibody rise (see Table 1).
[b] Diameter (millimeters) in duration before challenge/diameter 3 days after challenge.
[c] At least one of following: influenza-like illness, virus isolation, or significant antibody rise (see Table 1).
[d] Considered a significant reduction.
[e] ND, Not done.

TABLE 3. *PHA response of volunteers' lymphocytes before and after influenza virus administration*

Volunteers	Inoculum	After attenuated vaccine			After virulent virus challenge		
		Evidence of infection	PHA response[a]		Evidence of infection	PHA response[a]	
			Before	After		Before	After
1	Vaccine	+	91.6	2.3[b]	–	105.0	31.2
2	Vaccine	+	60.2	9.2[b]	–	115.8	52.7
3	Vaccine	+	62.8	5.8[b]	–	24.0	30.0
4	Vaccine	+	49.8	4.4[b]	–	49.9	59.2
5	Vaccine	–	52.3	24.5	–	100.9	14.6
6	Vaccine	+	118.3	8.2[b]	–	179.4	50.4
7	Vaccine	–	24.3	10.9	–	25.4	18.2
8	Vaccine	+	85.6	10.4	–	153.3	104.2
9	Vaccine	–	51.2	56.4	+	85.4	41.8
10	Vaccine	–	50.5	57.7	–	108.8	111.5
11	Vaccine	+	132.3	7.9[b]	–	44.9	38.0
12	Vaccine	–	109.2	66.4	–	75.6	87.7
13	Vaccine	+	89.2	5.1[b]	–	20.6	31.8
14	Placebo	–	20.7	84.0	+	129.5	10.3
15	Placebo	–	93.4	240.9	+	117.1	4.5[b]
16	Placebo	–	85.6	171.2	–	44.6	119.3
17	Placebo	–	47.6	18.5	+	63.8	20.2
18	Placebo	–	55.6	54.3	+	101.5	5.8[b]
19	Placebo	–	60.6	66.2	+	65.4	31.2
20	Placebo	–	24.7	59.9	+	31.5	7.1[b]
21	Placebo	–	51.4	90.6	+	119.5	93.6
22	Placebo	–	59.0	49.1	–	42.9	27.5
23	Placebo	–	28.5	80.1	+	78.0	28.1
24	Placebo	–	45.7	91.9	+	58.8	22.3
25	Placebo	–	22.6	41.9	+	94.4	6.1[b]
26	Placebo	–	70.5	44.2	+	38.3	7.1[b]
27	Placebo	–	61.2	51.9	+	106.6	20.6

[a] Stimulation ratio (see Materials and Methods).
[b] Considered a significant reduction.

attenuated vaccines to mumps (17), yellow fever (7), and type 1 poliovirus (4) have been shown to suppress the tuberculin reaction, and measles vaccine and vaccinia have been shown to suppress the histoplasmin skin test (16).

In vitro studies have shown that measles virus incubated with lymphocytes from tuberculin-sensitive children suppresses their blast transformation by tuberculin PPD (28). The lymphocytes from one of the patients were incubated with measles virus and PHA, and normal blastogenesis resulted. Infants with congenital rubella, although having no gross defect in antibody production, have a depressed lymphocyte response to PHA stimulation (1, 2, 9, 22, 23, 29). Furthermore, normal lymphocytes treated in vitro with rubella virus have a depressed response to PHA. Mumps, Newcastle disease, and poliovirus have also been shown to inhibit the in vitro response of normal lymphocytes to PHA (9, 23, 28).

Immunosuppression during viral infections may be a permissive factor in carcinogenesis and may also cause rapid progression of an already present tumor. It has been shown that various forms of immunosuppression, including iatrogenic ablation, graft versus host disease, the neonatal state, or certain viral infections, enhance the appearance of viral-induced tumors in the laboratory animal (15). There is ample evidence that the immunosuppressed state in man is associated with a higher incidence of malignancy. The relationship of the primary immunodeficiency disorders and malignancy has been summarized by Good (13). There is a tenfold increase in the incidence of malignancies arising de novo in patients under immunosuppressive regimen for organ transplantation (12, 19, 31).

The purpose of the present study was to evaluate the effects of a live attenuated influenza vaccine and virulent influenza infection on cutaneous delayed hypersensitivity and in vitro lymphocyte response to the mitogen PHA. There have been two previous studies evaluating the effect of acute influenza infection on

cutaneous delayed hypersensitivity. The first study had to rely solely on clinical means in diagnosing influenza as serologic and virologic assays were not in existence at that time (5). A recent study by Reed et al. has shown cutaneous anergy to be present in all seven of their serologically proven influenza cases (27). The present study is the first to evaluate immunosuppression after administration of an attenuated influenza vaccine, and also immunosuppression after deliberate challenge with virulent influenza virus. Of eight volunteers who demonstrated infection after administration of the attenuated vaccine, four had a diminution in their skin test response, three of the four disappearing completely. Of 13 volunteers deliberately infected with virulent influenza virus, 8 demonstrated a diminution in their skin test response.

The study by Reed et al. cited above indicated that patients' lymphocytes had a normal PHA response at a time when the patients were demonstrating skin anergy. Another recent study, however, showed suppression of the PHA response during naturally occurring influenza virus infection (26). In the present study, of the 21 volunteers who were infected with either attenuated or virulent influenza virus, 12 had suppression of their PHA response. Although most of the volunteers demonstrated suppression of T-cell function, there was no evidence of a similar suppression of B-cell function. This was assessed by measuring serum antibody to an unrelated antigen before, during, and after influenza virus infection. No change in antibody titer was noted.

The significance of the suppression of cell-mediated immunity noted in this and the other studies is that: (i) if common viral infections are shown to induce an immunosuppressed state for a significant period of time, an important factor in carcinogenesis might be elucidated; (ii) this may have implications with respect to the development and use of live virus vaccines, since it might be harmful to deliberately induce an immunosuppressed state, as might be occurring in children and adults who receive a variety of attenuated vaccines; (iii) immunosuppression during influenza may enable endogenous latent infections such as tuberculosis to reactivate; (iv) immunosuppression during influenza may cause a progression of currently active infections normally contained by cell-mediated immunity, e.g., measles causes a progression of tuberculosis (24); and (v) immunosuppression during influenza may explain in part the fact that influenza is associated with an increased incidence of bacterial pneumonia.

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