Antibody-Associated Lymphotoxin in Acute Infection

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A b s t r a c t Five pediatric patients who were known
to be previously healthy acutely developed lymphopenia
during various viral or mycoplasma infections. In one
case, fatal generalized varicella occurred and in another,
severe toxic epidermal necrolysis ensued.

To further investigate this phenomenon, a study was
done to determine the incidence of and elucidate the
pathogenesis of lymphopenia occurring during the acute
phase of viral or mycoplasma infections. Acute and con-
valescent sera from patients with viral or mycoplasma
infection and children immunized with live measles virus
were screened for lymphocytotoxic activity against nor-
mal lymphocytes by the microcytotoxicity method of
Terasaki and McClelland (1). Sera with lymphocyto-
toxic activity were found in 15 of 48 cases of viral in-
fections, 4 of 22 mycoplasma infections, and 1 of 11
measles virus immunized persons. All those who had
sera which were cytotoxic to lymphocytes in vitro had
lymphopenia. The lymphocytotoxic activity resided in
19S fractions in 8 of 11 positive sera while the remaining
3 had activity both in 19S and 7S fractions and could
be completely removed by absorption with antilight
chain antiserum. The cytotoxic activities were all com-
plement-dependent and were greater at 37°C than at 4°C.

The significance of acute acquired immunologic de-
ficiency due to the development of antibody-associated
lymphotoxin (AbAL) during acute infections is dis-
cussed and five cases having more severe clinical mani-
festations are presented (Appendix).

INTRODUCTION

Recently the presence of "cold lymphocytotoxin" was
shown in patients with infections such as rubella, rubeola,

and infectious mononucleosis (2). Many previously de-
scribed cytotoxins related to diseases were shown to be
most active at cold temperatures when tested in vitro
(2). It is unknown whether such lymphocytotoxins
could have any direct effect at normal body temperature.
In addition, some cytotoxins effective at 37°C have been
observed (3–6).

We recently observed four cases in our hospitals that
suddenly developed lymphopenia (defined as < 1,500/
mm³) and severe clinical illness in the wake of a viral
or mycoplasma infection. In a fifth, a vague febrile ill-
ness preceded by 10 days a terminal varicella infection.
These observations prompted us to investigate the inci-
dence and mechanism of lymphopenia associated with
some infectious agents.

METHODS

Source of sera. In addition to the five original cases, sera
were obtained from 48 cases of various viral and 22 of
mycoplasma infections. Diagnosis was verified by isolation
of the agents and demonstration of at least a fourfold rise
in antibodies in convalescent sera. Acute sera were collected
within 7 days after the onset of illness and convalescent
sera were collected between 15–25 days after the onset of
illness. Four age-matched control sera were obtained for
each test sample. Two were obtained from cases with febrile
illnesses without elevation of any viral antibodies in sub-
sequent sera and two were from cases with various viral ill-
nesses and subsequent elevation of antibody titers but which
did not develop lymphopenia. None of these control cases
were shown to have bacterial infection. The white blood
cells were taken at the time when acute and convalescent
sera were obtained except in two cases. Sera were also
obtained from 11 individuals 2 wk after immunization with
live measles virus. 22 healthy adults were also selected as
normal controls.

Detection of lymphocytotoxicity. Cytotoxicity was tested
by Terasaki's microdroplet method (1). Test lymphocytes,
human sera (in some cases 19S or 7S fractions) and rabbit
complement were added in that order to disposable plastic
trays and incubated at 4°C for 3 h or 37°C for 1 h. (Sam-
ples of the same complement source were used throughout
the experiment.) Both acute and convalescent sera were
tested for cytotoxicity. The lymphocytes for testing were
obtained from randomly selected healthy individuals; in

TABLE I

Infectious Agents Associated with Antibody-Associated Lymphocytotoxin (AbAL)

Agents	No. of cases studied	No. of cases with lymphopenia	No. of cases with AbAL
H. simplex	7	2	2
adenovirus	6	0	1
rubella	13	4	4
mumps	6	2	2
influenza B	4	1	1
measles	2	2	2
RSV*	8	2	2
varicella	1	1	1
mycoplasma	22	4	5
unknown	1	1	1
measles‡	11	1	1

* Respiratory syncytial virus.

‡ Immunized with live attenuated virus.

some cases autologous lymphocytes were tested also. Two panels of six cells each, obtained from a total of 12 donors, were used during the study. The criterion for positivity in our test was that three or more individual members of the donor lymphocyte panels showed more than 20% cytotoxicity as judged by eosin dye exclusion. Antilymphocyte serum was used as a positive control and always revealed more than 90% cytotoxicity to all donor cells. Each test was done in duplicate and was repeated at least three times. The result was read independently by two investigators and always agreed from time to time and between the investigators. The identity of the samples, whether lymphopenic or not, was unknown at the time of the reading. Sera from age-matched controls and 22 healthy individuals (12 males and 10 nulliparous women) were uniformly negative. It was noted that loss of cytotoxic activity occurred after repeated freezing and thawing of acute serum samples. The specific virus with which the patient was infected was added to the test mixtures in an attempt to restore the activity. This was done in two ways: (a) live virus was first incubated with normal lymphocytes for 4 h at 37°C, the cells then washed, and the test was conducted as described above; (b) live virus was incubated with the appropriate test serum before addition to the test lymphocyte system. The test lymphocytes in this system were adjusted to a concentration of 1.0×10^6 cells/milliliter in minimal essential medium (MEM) with 10% fetal calf serum, penicillin, and streptomycin. The titer of the virus suspensions were adjusted to approximately 10^{-4} tissue culture dose (TCD$_{50}$)/0.1 ml before testing. No difference resulted from the order of addition. Virus used for testing included: rubella, mumps, influenza B, measles, varicella, Herpes simplex, and respiratory syncytial virus. The control systems for the above tests included: (a) virus and complement only, (b) virus and normal human serum only, (c) virus and sera of infected but nonlymphopenic patients plus complement, (d) serum from infected patients plus a virus different from the infecting agent plus complement, (e) virus alone, (f) complement alone. Negative results were consistently obtained with these controls.

Separation of sera into 7S and 19S fractions was accomplished by sucrose density gradient separation in a Beck-

man Model L 65B^R ultracentrifuge (Beckman Instruments, Inc., Palo Alto, Calif.) (7).

HL-A antigens and antibody. The HL-A antigens of the testing lymphocytes were determined by Dr. T. Iha of the State Laboratory of Hygiene, Madison, Wisconsin, and HL-A antibody screening on the sera were performed by Dr. E. Yunis of the Immunology Division, University of Minnesota Hospital, Minneapolis, Minnesota. This test utilizes a test panel of lymphocytes obtained from 20 donors. Immunoabsorbents were prepared by conjugation of anti-light chain antiserum to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) by the method of Cuatrecasas, Wilchek, and Anfinsen (8). Immunoglobulins were absorbed from test sera by exposure to the immunoabsorbent for 60 h at 4°C using constant agitation. Quantitation of the serum immunoglobulins by single radial diffusion (9) revealed that 96% of IgG, 90% of IgA, and 99% of IgM were removed. Simultaneous quantitation of albumin showed no change indicating the specificity of the immunoabsorption.

Specificity of the lymphocytotoxic effect. In order to test the specificity of the lymphocytotoxic effect, several human cell lines were utilized as target cells for 10 positive sera. Inadequate amounts precluded testing of all positive samples. The cell lines employed were HeLa, Hep-2, Wis-L (p-7, fetal epithelial cells), Wis-L (p-24, fetal fibroblasts), and WI-38 (p-25, lung fibroblasts). Cell concentrations were adjusted to 1×10^6 cells/milliliter for cytotoxicity tests. Viruses, serum samples, and complement were added in order as described above in the detection of lymphocytotoxicity. Subsequently, those cells were subcultured to detect any possible deleterious effect on the formation of monolayers.

Human granulocytes from peripheral blood were isolated by Böyum's method (10) from three of the donors and were also tested for cytotoxicity. Human peripheral lymphocytes from the same donors after incubation with sheep red blood cells were separated by Ficoll Hypaque (Pharmacia Fine Chemicals, Inc.) density gradient into subpopulations of rosette-forming cells (RFC) and nonrosette-forming cells (n-RFC) [1] and each group was used as target cells for the above 10 positive sera.

Appropriate controls were prepared for each experiment as described above and all the tests were performed at 37°C.

RESULTS

Screening among 48 cases with viral infections showed that 15 cases had lymphopenia while 4 of 22 cases with mycoplasma infection developed lymphopenia (Table I). No single agent seems more prone to induce lymphopenia. All of those cases which developed lymphopenia showed lymphocytotoxic activity against normal lymphocytes in their acute phase sera (Table II). Five cases (Appendix) from whom lymphocytes were obtained and tested against their own sera showed autocytotoxicity. Two cases which had peripheral blood lymphocyte counts of more than 1,500/mm^3 showed only weak cytotoxic activity in their sera.

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[1] *Abbreviations used in this paper:* AbAL, antibody-associated lymphotoxin; CMI, cell-mediated immunity; n-RFC, nonrosette-forming cells; RFC, rosette-forming cells.

TABLE II

Clinical Data of Patients with Lymphopenia

Case	Age	Sex	Offending microorganism	Clinical symptoms	Total leuko-cytes	Absolute lym. count	Cytotoxicity vs. panel of 6 different donor lymphocytes*	C. F.	Ab‡
1	9	M	mumps	parotitis	11,700 8,600§	1,404 1,750	++, ++, ++, ++, −, ++, −, −, −, −, −, −,		<1:8 1:64
2	14	M	RSV‖	pneumonitis	6,800 9,300	1,360 1,860	++, ++, +, ++, −, −, −, −, −, −, −, −,		1:8 1:64
3	12	F	H. simplex	stomatitis, skin infection	4,000 12,000	1,480 3,560	++, ++, +++, +++, ++, ++, −, −, −, −, −, −,		<1:8 1:64
4	2	M	mumps	parotitis	9,300 8,600	1,302 2,150	++, ++, +, +, −, −, −, −, −, −, −, −,		1:16 1:128
5	11 mo	M	varicella	skin infection	3,990 9,800	1,037 2,250	−, −, +, ++, ++, ++, −, −, −, −, −, −,		1:16 1:128
6	10	M	influ. B	pneumonitis	12,250 10,200	945 3,300	−, ++, −, +, ++, ++, −, −, −, −, −, −,		<1:8 1:64
7	10	F	measles, Candida, Staph aureus	scalded skin syn.	12,300 10,700	750 4,000	+, ++, ++, +, ++, +, −, −, −, −, −, −,		1:8 1:256
8	12	M	H. simplex mycoplasma	Stevens Johnson syn., pneumonitis	7,300 6,500	500 2,990	++, ++, ++, ++, ++, ++, −, −, +, −, +, −,	Herpes Mycoplasma	1:32 1:256 1:16 1:128
9	6	M	measles	exanthema	4,000 8,600	480 1,920	+, ++, ++, +, ++, −, −, −, −, −, −, −,		<1:8 1:32
10	3	F	RSV	bronchiolitis	3,900 8,700	1,499 1,850	−, ++, ++, ++, +, −, −, −, −, −, −, −,		<1:8 1:64
11	8	M	rubella	exanthema	5,000 7,800	1,350 2,200	−, ++, −, ++, +, ++, −, −, −, +, −, −,		<1:8 1:64
12	13	M	rubella	exanthema	4,500 6,700	1,125 1,650	+, +, +++, ++, ++, +, −, −, +, −, −, −,		<1:8 1:256
13	17	F	rubella, herpes	stomatitis, UTI	7,680 6,400	230 1,560	++, +, +, +, +, +, −, −, −, −, −, −,	Rubella	<1:8 1:128
14	10	M	?¶ varicella	disseminated varicella inf. (deceased)	9,300	970	++, +, +, +, ++, +,		
15	12	M	rubella mycoplasma	erythema pneumonitis	7,800 8,000	156 1,750	++, ++, ++, +, ++, −, −, −, −, −, −, −,	Rubella Mycoplasma	<1:8 1:128 1:16 1:256
16	13	M	mycoplasma	URI	8,300 8,900	897 3,115	+, ++, ++, +, −, −, −, −, −, −, −, −,		1:16 1:256
17	15	F	mycoplasma	pneumonitis	5,000 7,300	700 1,505	+, ++, ++, −, +++, +, −, −, −, −, −, −,		1:32 1:256
18	20	M	mycoplasma	pneumonia	15,300 9,800	918 2,250	+, +, ++, ++, −, −, −, −, −, −, −, −,		1:8 1:128
19	31	F	mycoplasma	URI	5,200 7,600	1,144 3,420	+, ++, ++, −, −, −, −, −, −, −, −, −,		1:16 1:128
20	2	F	adenovirus	fever	5,500 8,000	1,650 2,050	++, ++, −, −, ++, +, −, −, −, −, −, −,		1:8 1:64
21	15	F	mycoplasma	pneumonitis	8,800 6,400	1,532 1,728	++, +, ++, +, −, −, −, −, −, −, −, −,		1:32 1:256

* Results arranged in same order for all patients; (−), no reaction; (+), 20% cytotoxicity; (++), 40% cytotoxicity; (+++), 60% cytotoxicity (++++), 80% cytotoxicity (see ref. 1).
‡ Complement-fixing antibody titre for the specific infectious agent.
§ Second line of each entry shows values obtained during convalescence.
‖ Respiratory syncytial virus.
¶ Preceding infection assumed; no definite agent recovered.
** Cases without lymphopenia but which showed cytotoxicity.

Of 11 cases immunized with live measles virus, only one showed cytotoxic activity at 37°C. However, four other cases showed cytotoxic activity at 4°C incubation in sera obtained 2 wk after immunization. Lymphopenia was not present. With the exception just mentioned, all the cytotoxic agents of the positive sera showed certain common characteristics: (a) optimal activity at 37°C, (b) complement dependency, and (c) ease of demonstration in acute phase sera but essentially nondetectable in convalescent sera (0 in 12, 1 + in 3).

Reactivation of the cytotoxic activity of the acute sera was observed after addition of viruses to test cells by either of the methods described above. These results are shown on Table III. It can be seen that increased cytotoxicity was obtained after addition of appropriate viruses in nearly all cases and frequently the return was equal to or greater than the original activity. Cytotoxicity could not be produced in any of the convalescent sera by the addition of virus.

In an attempt to further define the nature of the serum factor(s), immunoglobulins were removed by incubation with an antilight chain immunoabsorbent. Absorbed sera did not show lymphocytotoxic activity, and the addition of viruses had no effect on reconstitution of the absorbed sera.

11 of the positive sera were separated into 7S and 19S fractions and the fractions were tested for cytotoxicity as described above. In all serums, the cytotoxicity seemed to reside in the 19S fraction and in only three sera was there activity in the 7S fraction.

None of the human tissue culture cell lines in suspension demonstrated cellular damage as shown by eosin dye exclusion tests during microcytotoxicity tests and further, there were no deleterious effects which prevented formation of monolayers. Treated cell lines were identical to controls over a period of a 72 h observation. In addition, no cytotoxic effect was demonstrable against granulocytes obtained from three normal donors.

When the cytotoxicity test was done on RFC's (T cells) and n-RFC's (B cells) (11–13) from peripheral blood of three donors, a significant cytotoxic effect from + to +++ was noted for RFC's (T cells) for all three donors by each of 10 positive sera. Only minimal cytotoxicity (maximum +1) was seen in 3 of 10 positive sera for n-RFC's (B cells). In addition, when positive serums were added to lymphocytes in a subcytotoxic concentration (1:20 final dilution), a 30–40% inhibition of sheep cell rosette formation was observed.

DISCUSSION

Lymphocytotoxins or lymphocytotoxic antibodies were described in sera from allografted animals several years ago (14, 15). In 1964 a satisfactory microdroplet method was developed (1) and it was soon established that lymphocytotoxin appeared in human sera in the course of various clinical conditions (2–6, 16–27).

Some of the previously described lymphocytotoxins showed cytolytic activity best at cold temperatures (15°C) and complement dependency. Some were associated with various autoantibodies (16) or with certain types of HL-A antigens (22). Others have been described in association with certain phases of different clinical diseases (2–6, 19). The clinical significance of the "cold toxins" at body temperatures, however, is not yet well understood, although it has been speculated that they might be biologically active in a way similar to cryoglobulins or cold autohemolysin anti-i (28). For reasons of clarity, we refer to our serum factor as antibody-associated lymphotoxin (AbAL). This avoids confusion with lymphocytotoxins which are lymphokines produced as part of a cell-mediated immune reaction (29).

Screening of cases whose specimens were referred for virological studies showed that 15 out of 48 had lymphopenia (absolute count of lymphocytes less than 1,500/mm³) during certain periods of acute infections. The lymphopenia in all cases was correlated with the presence of AbAL and the return to normal (usually within 2–3 wk) was associated with loss of lymphocytotoxic activity of the sera. As indicated in Table II, there were only two patients who did not have lymphopenia by the above criteria, yet their sera had cytotoxic activity. This might be due to the fact that their counts were not taken until several days after the acute sera had been drawn. Most of the convalescent sera showed only weak or no cytotoxic activity.

The fact that diminution of the activity of the sera was observed after absorption with antilight chain antibody suggested that the cytotoxic factor was immunoglobulin in nature. Further studies demonstrated that the cytolytic action could be produced by adding virus, serum containing its specific antibody and complement to the testing lymphocytes. The gradual loss of this cytotoxic activity after the freezing and thawing of the positive sera could be interpreted as due to either the instability of antibody or possible destruction of virus. Addition of appropriate virus restored this loss. The mechanism for this phenomenon remains obscure, however. It is possible that viral antigen-antibody complexes were disrupted by freeze-thaw and new virus was required for new complex formation. Perhaps new virus altered the target lymphocytes, rendering them more susceptible to lysis. The fact that only the virus which originally infected the patient was effective in this regard rules out a nonspecific cytotoxic effect of viral origin. These observations suggest that a number of unique interactions between host lymphocytes, humoral antibody, and infecting virus may be required for lym-

TABLE III

Restorative Effect of Virus on Lymphocytotoxicity

Case	Virus	Condition	Donor 1	2	3 (degree of cytotoxicity)	4	5	6
1	mumps	A*	++‖	++	++	++	−	++
		F − T‡	−	−	−	−	−	−
		F − T + V§	+	++	−	−	−	+
2	RSV¶	A	++	++	+	++	−	−
		F − T	−	−	−	−	−	−
		F − T + V	+++	+	−	−	−	−
3	H. simplex	A	++	++	+++	+++	++	++
		F − T	−	−	−	−	−	−
		F − T + V	++	++	+	+	−	−
4	mumps	A	++	++	+	+	−	−
		F − T	−	−	−	−	−	−
		F − T + V	−	+	−	+	++	−
5	varicella	A	−	−	+	+	+	+
		F − T	−	−	−	−	−	−
		F − T + V	−	−	−	+++	++	+
6	influ. B	A	−	++	−	+	++	++
		F − T	−	−	−	−	−	−
		F − T + V	−	+++	−	+	+	+++
7	measles	A	++	++	+	++	+	−
		F − T	−	−	−	−	−	−
		F − T + V	++	++	−	++	++	−
8	mycoplasma	A	++	++	++	++	++	++
		F − T	−	−	−	−	−	−
		F − T + V	+++	++	−	+	−	+
9	measles	A	+	++	++	+	++	−
		F − T	−	−	−	−	−	−
		F − T + V	−	++	+	−	+	−
10	RSV	A	−	+	+	+	+	−
		F − T	−	−	−	−	−	−
		F − T + V	−	−	+	+	−	−
11	rubella	A	−	++	−	++	+	++
		F − T	−	−	−	−	−	−
		F − T + V	−	+	−	++	−	+
12	rubella	A	+	+	+++	+	+	+
		F − T	−	+	+	−	−	−
		F − T + V	−	+	+	+	−	−
13	H. simplex	A	++	+	−	+	+	+
		F − T	−	−	−	−	−	−
		F − T + V	+	−	−	+	−	−
14	varicella	A	++	+	+	+	++	+
		F − T	−	−	−	−	−	−
		F − T + V	++	+	++	+++	++++	+++
15	mycoplasma	A	++	++	+	+	++	+
		F − T	−	−	−	−	−	−
		F − T + V	+	+	−	−	−	−

* A, fresh serum specimen.

‡ F − T, serum after freeze and thaw five times.

§ F − T + V, virus added to serum after freeze and thaw.

‖ +, 20% cytotoxicity, ++++, >80% cytotoxicity; (−), no reaction.

¶ Respiratory syncytial virus.

phopenia to result. No single agent seems more prone to induce lymphopenia.

We also demonstrated that the cytotoxic activity mainly resides in the 19S fraction of the sera; three sera also showed some activity in the 7S fraction. The convalescent sera failed to demonstrate significant cytotoxic activity. Additionally, the possibility of a "blocking factor" in the convalescent sera has not been ruled out. It is possible that IgM was the major antiviral antibody in the acute phase while in the convalescent phase IgG was predominant.

At the time when the cytotoxic agent was demonstrable in the serum sample, titers of complement-fixing antivirus antibody were $1:8$ or less. Also, during convalescence, when complement-fixing titers had risen fourfold or more, virus-serum cytotoxicity effects had disappeared. Thus, the agent seems different from classic antibody as detected by usual complement-fixing methods. However, the sedimentation characteristics and the association with light chain determinants lead us to believe that the agent is an antibody.

Human cell lines other than lymphocytes were also tested in the same cytotoxicity system but there was no cytotoxic effect seen among those cell lines nor with granulocytes. In preliminary tests, the cytotoxic activity seemed to be greater for T lymphocytes than B lymphocytes. Further effects upon cell-mediated immunity (CMI) function are still under study but if these results are substantiated they may bear upon the transient anergy noted in various viral infections (30).

There is a form of immunological deficiency in humans reported by Kretschmer, Janeway, and Rosen (26) in which a patient was noted to have dysgammaglobulinemia and also lymphopenia due to the presence of lymphocytotoxic antibody. As expected, she manifested failure of lymphocyte-dependent functions including allograft rejection, delayed hypersensitivity, and immunologic memory. It is interesting to note that her serum lymphotoxic antibody was associated with γ-A globulin and that bouts of recurrent profound lymphopenia followed infections. We have observed a similar course of events in a patient with thymic deficiency and lymphopenia. Viral infections associated with AbAL formation resulted in complete absence of lymphocytes and on at least two occasions, Pneumocystis pneumonia. After successful therapy the lymphocyte count returned to its preillness state and AbAL was no longer demonstrable.[2]

The cytotoxic activity described in our studies was not due simply to HL-A antibody activity. Our cases differ from individuals possessing HL-A antibodies in a number of ways. There was no history of previous

transfusion or allogeneic stimulation. Serum cytotoxic activity was present only transiently—i.e., in the acute stage but not in convalescent sera. Addition of viruses restored cytotoxic activity. Finally, no HL-A antibody activity could be demonstrated in our cytotoxic sera when tested against a panel of 20 donors. The number of donors is admittedly small, however.

In two cases, the development of lymphopenia resulted in severe clinical symptoms (Case 14 and 7 of Table II); both patients had been known to be healthy previously. Case 14 succumbed to disseminated varicella. He had had live virus immunizations previously without any complication. Postmortem studies showed the depletion of small lymphocytes from the lymph nodes. Unfortunately, the thymus was not studied and the cause of the previous infection was not clear. Case 7 apparently was exposed to measles resulting in elevation of measles antibody, although she did not demonstrate clinical disease. While she was having lymphopenia she was infected with Candida albicans and a Staphylococcus aureus of an unusual phage type. She developed the scalded skin syndrome which is extremely rare in this age group. The restriction of the scalded skin syndrome to the very young in human and experimental situations suggests some immunologic compromise as important in its etiology. In the two cases just mentioned, it is postulated that AbAL induced sufficient impairment of immunity to allow the unusual expression of a superimposed infectious agent. Recently, Dudding and his associates (31) reported three fatal cases of pneumonia associated with adenovirus type 7. The patients were previously in good health but succumbed to death after a brief fulminant illness. One striking feature of those cases was persistent lymphopenia. Although the superimposition of other bacterial infection or other viral infections was not clear in their cases, the clinical picture was very similar to acute acquired immunologic deficiency which occurred in our two cases.

Three other patients had less severe illnesses as compared to the above cases and recovered. All their lymphocyte counts returned to normal with the disappearance of AbAL except one patient, Case 15, who had sustained lymphopenia for nearly 2 mo. It was not clear whether this patient had persistence of virus-antibody complex in the circulation or chronic viremia, but his course was unusually prolonged. Fortunately, he recovered without any further clinical complications.

Only one case developed AbAL after live measles virus immunization among 11 cases studied. She had an uneventful clinical course throughout the observation. Four cases, however, did have lymphocytotoxic factor optimally active at 4°C. This finding is consistent with the observations of Kreisler, Hirata, and Terasaki (19). Unfortunately, it was not possible to

compare the effect of natural measles infections with live virus vaccination as regards the development of AbAL.

Host responses to viral infections have been reviewed extensively recently (30). Some important biological phenomena associated with viral infections such as tissue injured mediated by antiviral antibody through a complement effector system (32), inhibition of in vitro lymphocyte blast transformation, abrogation of delayed type hypersensitivity (30) are well established by many studies. The basic mechanism for such phenomena remains unclear. Factors such as the possible existence of virus receptors on cell membranes as well as the role of HL-A antigen in humans may play an important role. By whatever mechanism, the destruction of target lymphocytes as a consequence of viral infection may set the stage for serious clinical disease.

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APPENDIX

Case 7, a 10-yr-old female, was admitted in a semicomatous state after a short febrile illness. She had a generalized skin eruption with whitish-gray papules and an extensive denudation with a positive Nikolsky's sign—a picture of scalded skin syndrome (toxic epidermal necrolysis). Candida albicans was cultured from the tongue and vagina. Skin cultures yielded Staphylococcus aureus, coagulase positive, phage type 187. There was no significant lymphadenopathy. Her absolute lymphocyte count was 750/mm³ on admission and 1,750/mm³ on the 4th hospital day. She was discharged 2 wk later after a complete recovery. She had been exposed to measles before the acute illness and acquired an elevated antibody titer without clinical disease.

Case 8, a 12-yr-old male, had a history of a febrile illness for a week followed by a generalized skin rash. He also had conjunctivitis, stomatitis, and left lower lobe pneumonia. His absolute lymphocyte count was 500/mm³ on admission and 2,576/mm³ on the 10th hospital day. He had an initial elevation of Herpes simplex antibody titer and mycoplasma complement-fixing titer was elevated during the recovery.

Case 13, a 17-yr-old female, had a prior mild febrile course for 7 days and then developed a high fever, abdominal pain, and stomatitis. Herpes virus was isolated from the lesion and urine culture grew micrococci. She was exposed to rubella without developing any rash but the antibody titer became elevated. Her absolute lymphocyte count was 230/mm³ and returned to 1,560/mm³ on the 4th hospital day.

Case 14, a 10-yr-old boy, was admitted in a state of shock with a history of minor symptoms for 10 days. Subsequently, he was found to have myocarditis, pericarditis, pleural effusion, hepatic disease, and possible central nervous system lesions. He also developed disseminated intravascular coagulopathy and expired on the 7th day. Postmortem findings revealed that he had disseminated varicella. The virus was isolated from the lung, spleen, and liver. His absolute lymphocyte count was 1,000/mm³ on admission and then dropped to 960/mm³. Unfortunately, histological study of the thymus was not done. Although he was previously well and his vaccination history was uneventful, he was known to be exposed to varicella during his early illness and he never developed skin manifestations.

Case 15, a 12-yr-old male, was admitted with a generalized maculopapular rash after a minor illness for 2 wk. Chest X ray showed viral pneumonitis. Profound lymphopenia with a range of 73/mm³–336/mm³ was noted and it was 2 mo before his count was up to normal. His sister had received a rubella vaccine before his illness and had an elevated titer of mycoplasma during the recovery. He did not have any clinical problems while he had prolonged lymphopenia.

These five cases were previously healthy and all had complete immunizations without any problems. Most of the immunological tests done during hospitalizations were normal except for slightly low IgM and complement in Case 14 and a low phytohemagglutinin transformation in Case 7.

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