

LYMPHOCYTE TRANSFORMATION INDUCED BY AUTOLOGOUS CELLS

VIII. Impaired Autologous Mixed Lymphocyte Reactivity in Patients with Acute Infectious Mononucleosis*

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A subpopulation of human bone marrow (B) lymphocytes stimulates the proliferation of autologous thymus-derived (T) lymphocytes (1, 2). This autologous mixed lymphocyte reaction (MLR)¹ has been shown to have two cardinal characteristics of an immune response, i.e., memory and specificity (3). Furthermore, the autologous MLR has been shown to provide a proliferative stimulus required for the generation of cytotoxic lymphocytes (4). More recently, generation of suppressor cell activity has been demonstrated during this reaction (5, 6). This suggests a potential immunoregulatory function for the autologous MLR.

Infectious mononucleosis (IM) is a viral disease often associated with transient autoimmune phenomena (7, 8). These studies were undertaken to correlate the autologous MLR with disease activity during IM. Patients with acute IM did not generate a normal analogous MLR during the acute phase of the disease. However, the autologous MLR returned to normal levels during convalescence from IM. The cellular basis of the defect in the autologous MLR was shown to be a result of the failure of B cells to stimulate autologous T cells.

Materials and Methods

Patients Studied. Nine patients with acute IM were studied. Patients were males and females between the ages of 18 and 27. Diagnosis was based upon presentation of fever, sore throat, lymphadenopathy, a positive heterophile index, and the presence of atypical lymphocytes in the peripheral blood. Patients were untreated at the time of the study. Healthy young subjects without evidence of prior immunity to rubella virus, as determined by hemagglutination inhibition (HI), were inoculated with live attenuated rubella virus, HPV-77 (Meruvax, Merck, Sharp and Dohme, Div. of Merck & Co., Inc., West Point, Pa.). The autologous MLR of these individuals was examined from 3 to 21 d after inoculation. All subjects developed HI antibodies to rubella 2-4 mo after inoculation.

Autologous and Allogeneic MLR Cultures. The autologous and allogeneic MLR cultures were established as previously described (1). Briefly, human peripheral blood T lymphocytes that

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¹ *Abbreviations used in this paper:* EBV, Epstein-Barr virus; HI, hemagglutination inhibition; IM, infectious mononucleosis; MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin.

formed rosettes with sheep erythrocytes were separated from non-T lymphocytes. Throughout this report we use the term B lymphocyte to identify the nonrosetting B-cell-enriched population of cells, recognizing that this preparation is heterogeneous containing B cells, null cells, K cells, and monocytes. 7% of the T-lymphocyte preparation and 93–98% of the B-lymphocyte preparation reacted with fluorescinated anti-human immunoglobulin antibody. 15–35% of the mononuclear cells in the B-lymphocyte preparation phagocytized latex particles. Culture wells contained 10^6 purified T lymphocytes and $1-2 \times 10^5$ irradiated (3,000 R) autologous or allogeneic B lymphocytes in 0.2 RPMI-1640 with 20% human serum from AB-type blood donors. Cultures were incubated 6 d. Thymidine incorporation was measured during the last 24 h of incubation. The average of the counts per minute of triplicate cultures is given.

Phytohemagglutinin Stimulation. Each culture well contained 0.2 ml of final culture medium, 1×10^5 T lymphocytes and, where indicated, 2 μ g phytohemagglutinin (PHA) (Burroughs-Wellcome Co., Research Triangle Park, N. C.). Cultures were established in triplicate, incubated for 4 d, and thymidine incorporation was measured during the last 24 h of culture.

Cryopreservation. The concentration of T or B lymphocytes was adjusted to $6-12 \times 10^6$ cells/ml in RPMI-1640 supplemented with 50% heat-inactivated human AB serum at 4°C. An equal volume of media that contained 20% dimethyl sulfoxide (Fisher Scientific Co., Pittsburgh, Pa.) was slowly added to the cell with constant mixing at 4°C. Cells were then aliquoted to polypropylene screw-cap vials. The vials were gradually cooled to -70°C and finally transferred to a liquid nitrogen storage unit. Cryopreserved cells to be used in assays were defrosted as follows: upon removal of vials from the liquid nitrogen storage unit, the contents were rapidly warmed to 37°C and diluted slowly with constant agitation with RPMI that contained 20% AB sera. This suspension was then centrifuged and resuspended in media. Cell recovery from freezing varied from 80 to 90% based on trypan blue exclusion assays.

Results

Autologous and Allogeneic MLR in Patients with Acute IM. T lymphocytes from normal subjects or subjects with IM were cultured with irradiated autologous or allogeneic B lymphocytes (Table I). The autologous MLR in patients with IM was severely and significantly ($P < 0.001$) depressed when compared with the autologous MLR in normal subjects. In contrast to the depressed autologous MLR, the allogeneic MLR in patients with IM was equal to the allogeneic MLR generated by healthy age-matched controls. Thus, T lymphocytes from these individuals respond normally to allogeneic B lymphocytes but poorly or not at all to autologous B lymphocytes. Although, B lymphocytes from eight patients with IM stimulated thymidine incorporation by allogeneic T lymphocytes less well ($32.5 \pm 7.0 \times 10^3$ cpm) than did B lymphocytes from eight normal subjects ($44.5 \pm 13.0 \times 10^3$ cpm), these results did not reach statistical significance ($0.05 < P < 0.1$). T lymphocytes from patients with IM cultured alone incorporated more thymidine than did T lymphocytes from normal individuals, but this difference was not statistically significant. As has been reported previously, the response to the T-cell mitogen PHA, was depressed during the acute phase of IM (Table I). The response of patients with IM was $\approx 30\%$ of the response of control subjects ($P < 0.01$). Because the patients with IM studied had a self-limited disease, we were next able to determine if the normal autologous MLR was restored during convalescence.

Recovery of the Autologous MLR during Convalescence. The autologous MLR was tested 4–16 wk after diagnosis when the patients had recovered from acute IM (Fig. 1). All seven patients had a significantly impaired autologous MLR at the time of diagnosis. In these patients the autologous MLR was depressed by $>85\%$. Nevertheless, six of the seven regained a normal autologous MLR within 16 wk of diagnosis. The

TABLE I
*Impaired Autologous MLR in Patients with IM**

T-lym- phocyte donor	Stimulus		
	Autologous B lymphocyte	Allogeneic B lym- phocyte	PHA
	<i>cpm/culture</i> × 10 ⁻³		
Normal (9)	16.3 ± 1.7	43.2 ± 11.5	48.2 ± 15.6
IM (9)	2.2 ± 0.8	43.6 ± 7.7	15.8 ± 5.6

* 10⁵ lymphocytes were incubated with an equal number of irradiated autologous or allogeneic B lymphocytes or 10 µg/ml PHA in 0.2 ml RPMI-1640 with 20% human AB serum. Tritiated thymidine incorporation was measured during the last 24 h of culture. Cultures were incubated a total of 6 d for MLR responses and 4 d for PHA responses. The background counts per minute (T lymphocytes cultured with irradiated autologous T lymphocytes) averaged 584 cpm for IM patients and 240 cpm for normal subjects. The data are presented as the mean ± SE.

autologous MLR in one individual increased during convalescence, but did not reach the normal range within 16 wk.

These studies did not distinguish between a failure of T cells to respond or a failure of B cells to stimulate an autologous MLR. This question was investigated by comparing the properties of cryopreserved lymphocytes obtained during the acute phase of the disease with lymphocytes obtained during convalescence.

Cellular basis of the impaired autologous MLR in patients with IM. To distinguish between a failure of T cells to respond or a failure of B lymphocytes to stimulate, the autologous MLR was measured in four patients during convalescence when their autologous MLR had returned to normal. At that time the capacity of acute-phase cryopreserved T cells to respond or acute-phase cryopreserved B cells to stimulate an autologous MLR was studied (Table II). As expected, in all four subjects, cryopreserved lymphocytes obtained during the acute phase of IM failed to generate an autologous MLR whereas convalescent lymphocytes demonstrated normal reactivity. Convalescent B cells stimulated both acute-phase and convalescent T cells equally. In contrast, acute-phase B cells did not stimulate either acute-phase or convalescent T cells. These results suggest that the impaired autologous MLR in patients with acute IM results from the failure of the B-cell population to stimulate autologous T-cell proliferation. It is important to note that cryopreservation of B cells did not impair their capacity to stimulate an autologous MLR. Thus cryopreserved T and B cells from two normal subjects were not altered in their ability to generate an autologous MLR (Table II).

The impaired stimulatory capacity of B cells could be a result of the loss of stimulatory determinants, the loss of a stimulatory subpopulation of cells, or the presence of suppressor activity in the acute-phase B-cell preparation.

Suppressor activity of the acute-phase B-cell preparation from one patient was assayed.² A sufficient number of acute-phase B cells from this individual were cryopreserved to permit a B-cell mixture experiment. Autologous T cells were cultured with convalescent B cells, acute-phase B cells, or a mixture of acute-phase and

² A second patient with IM has been studied with the same results found.

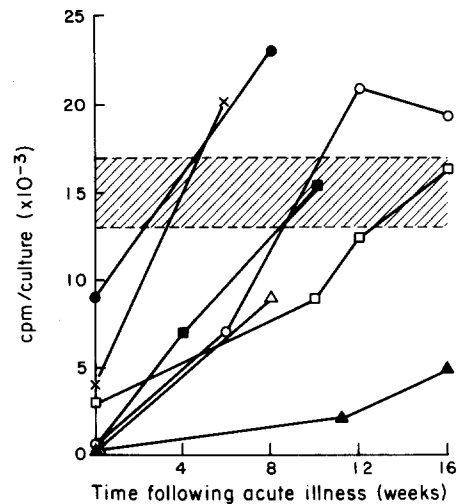


FIG. 1. Recovery of the autologous MLR during convalescence from acute IM. The autologous MLR was measured at the time of diagnosis and at intervals up to 16 wk after diagnosis in seven patients. 1×10^5 T lymphocytes were incubated with 1×10^5 autologous irradiated (3,000 rad) B lymphocytes for 6 d. Thymidine incorporation was measured during the last 24 h. Average counts per minute of triplicate cultures are given. The hatched area represents the mean \pm SE of seven healthy control subjects. The seven individual patients are indicated by the symbols \times , Δ , \blacktriangle , \square , \blacksquare , \bullet , and \circ .

convalescent B cells (Table III). Equal numbers (1×10^5) of acute-phase and convalescent B cells were mixed just before their addition to 1×10^5 responding T cells. Acute-phase B cells from this patient did not suppress the proliferative response of T cells cultured with convalescent B cells, although these acute-phase B cells failed to stimulate an autologous MLR.

Lymphocytotoxic antibodies and immune complexes reported to occur in acute IM might impair the autologous MLR. Preincubation of the lymphocytes from subjects with IM under conditions that usually clear the lymphocyte surface of these ligands did not increase the stimulatory capacity of these cells. However, data obtained by incubating normal autologous T and B lymphocytes in sera from patients with acute IM demonstrated statistically significant inhibition of the autologous MLR. Thus, in six separate experiments the autologous MLR was depressed 49% ($P < 0.05$) in the presence of serum from patients with acute IM as compared with serum from normal individuals. In contrast, allogeneic MLR and the PHA response were not significantly depressed (2 and 15%, respectively) by sera from patients with acute IM.

Normal Autologous MLR in Subjects Inoculated with Live Rubella Vaccine. The impaired autologous MLR in patients with acute IM might reflect the effect of viral infection. The effect of inoculation with attenuated rubella virus on the autologous MLR was measured in four females and one male. These five subjects without evidence of prior immunity to rubella were inoculated with live attenuated rubella vaccine (HPV-77). 3–21 d later, autologous MLR were performed. Three of the five subjects had symptoms of viral infection during this period. The autologous MLR was normal in all the subjects studied (Table IV) and was not significantly different from that observed in normal individuals (Table I). In contrast, the response to stimulation with PHA was significantly impaired ($P < 0.01$) 7 and 14 d after inoculation of live rubella

TABLE II
*Cellular Basis of the Impaired Autologous MLR in Patients with IM**

Subject	Responding T cell	Stimulating B cell	
		Cryopre- served	Fresh
<i>cpm/culture × 10⁻³</i>			
Patient A	Acute phase	0.7	6.5
	Convalescent	0.3	5.1
Patient B	Acute phase	1.3	13.3
	Convalescent	6.6	20.8
Patient C	Acute phase	0.1	14.7
	Convalescent	0.1	9.5
Patient D	Acute phase	0.3	12.8
	Convalescent	0.1	18.9
Control X	Cryopreserved	14.2	15.6
	Fresh	14.0	17.6
Control Y	Cryopreserved	10.9	12.3
	Fresh	12.7	12.4

* 10^5 T lymphocytes were incubated with 10^5 irradiated autologous B lymphocytes in 0.2 ml RPMI-1640 with 20% human AB serum for 6 d. Tritiated thymidine incorporation was measured during the last 24 h of incubation. T and B cells from patients with acute IM and from healthy controls were cryopreserved in liquid nitrogen for the same period of time. When the autologous MLR of patients had returned to normal, cryopreserved T and B cells (used as responding or stimulating cells, respectively) were cultured together or with freshly isolated autologous T or B cells. Cryopreserved cells were obtained from patients with acute IM. Fresh cells were obtained from these patients during convalescence.

vaccine. Subjects were subsequently retested for immunity to the rubella virus and were found to have developed HI antibodies.

Discussion

Transient depression of both cell-mediated and humoral immunity follows many viral infections (9). In 1908, Von Pirquet (10) reported that a child with measles showed a transient depression of the tuberculin skin reaction. Brody and McAlister (11) reported in 1964 that the tuberculin reaction was transiently depressed after administration of attenuated or killed measles (rubeola) vaccines. Lymphocytes from patients with a variety of viral diseases have a depressed response to PHA and other mitogens (12-16).

Infectious mononucleosis follows infection with the Epstein-Barr virus (EBV). Patients with IM have been found to have depressed cell-mediated immunity manifested by a loss of cutaneous hypersensitivity to tuberculin *in vivo* (17, 18) and a depressed response to PHA (15) and to allogeneic lymphocytes (19) *in vitro*.

The studies reported here demonstrate that the autologous MLR is impaired in patients with acute IM. The normal autologous MLR returns during convalescence. It is possible that convalescence may be associated with the reestablishment of a normal autologous MLR. It is of note that in patients in whom convalescence from EBV infection is very much prolonged, profound alterations in lymphocyte function have been observed (20, 21).

Our studies examined the cellular basis of the impaired autologous MLR. We

TABLE III
*Failure to Demonstrate Suppressor Activity in a B-Cell Preparation from a patient with IM**

Subject	Responding T lymphocyte	Stimulating B lymphocyte		
		Cryopreserved	Fresh	Cryopreserved and fresh
		<i>cpm/culture</i> × 10 ⁻³		
Patient D	Acute phase	0.6	7.8	7.9
	Convalescent	0.6	8.7	6.1
Control Y	Cryopreserved	13.1	12.8	12.9
	Fresh	13.6	14.5	10.1

* 10⁵ T lymphocytes were incubated with irradiated autologous B lymphocytes in 0.2 ml RPMI-1640 with 20% human AB serum for 6 d. Tritiated thymidine incorporation was measured during the last 24 h of incubation. T and B cells from patients with acute IM and from healthy controls were cryopreserved in liquid nitrogen for the same period of time. When the autologous MLR of patients had returned to normal, cryopreserved T and B cells (used as responding or stimulating cells, respectively) were cultured together or with freshly isolated autologous T or B cells. The cryopreserved cells were obtained from patients with acute IM. Fresh cells were obtained from the same patients during convalescence. Stimulating B lymphocytes were either 2 × 10⁵ cryopreserved cells, 2 × 10⁵ convalescent cells, or 1 × 10⁵ cryopreserved + 1 × 10⁵ convalescent cells.

TABLE IV
*T-Lymphocyte Responses of Humans Inoculated with Rubella Vaccine**

T-lymphocytes from vaccinated donors (Days after inoculation)	Stimulus	
	Autologous B lymphocyte	PHA
	<i>cpm/culture</i> × 10 ⁻³	
3 d (3)	16.1 ± 4.1	38.4 ± 9.6
7 d (4)	12.9 ± 4.0	17.9 ± 4.3
14 d (5)	14.4 ± 4.1	21.7 ± 7.9
21 d (4)	15.1 ± 2.0	44.7 ± 13.2

* 10⁵ T lymphocytes were incubated with 10⁵ irradiated autologous B lymphocytes or PHA (10 µg/ml) in 0.2 ml RPMI-1640 with 20% human AB serum for 6 or 4 d, respectively. Tritiated thymidine incorporation was measured during the last 24 h of culture. Counts per minute given are the mean ± SE. Subjects inoculated with live rubella vaccine lacked detectable immunity to virus before inoculation and developed detectable HI antibodies upon retesting 2-4 mo after inoculation.

found B lymphocytes from patients with acute IM do not stimulate proliferation of autologous T lymphocytes. No evidence for suppressor activity in the B-cell preparation was demonstrated. In contrast to the impaired stimulatory capacity of B lymphocytes, T lymphocytes from patients with IM respond normally to autologous and to allogeneic B lymphocytes. Furthermore, we found, as reported previously, that the response to PHA is impaired in patients with acute IM. During convalescence, the PHA response returns to normal. In contrast, the autologous MLR was not depressed in five patients inoculated with live rubella vaccine. We did confirm the impaired response to PHA of lymphocytes from subjects inoculated with rubella (16). Thus, an impairment in the autologous MLR is not a feature of all viral infections.

The immunoregulatory function of the autologous MLR has been suggested by a

number of investigators (1, 6). The impaired autologous MLR in patients who manifest autoimmune phenomena support this hypothesis (22, 23). Additional support comes from the finding that T cells activated in the autologous MLR express suppressor activity. Thus, cells generated in the autologous MLR suppress T-cell proliferation and the generation of cytotoxic T cells (6) and the induction of plaque-forming cells (24). Recently, Sakane and Green (5) have reported that suppressor cells induced by concanavalin A are drawn from the population of T cells activated in the autologous MLR.

As the normal autologous MLR may be an important means of generating suppressor cells, the appearance of autoantibodies in IM may be related to the impaired MLR in these patients. Anti-i, the main cause of autoimmune hemolysis that occasionally complicates IM, autoantibodies to B and T lymphocytes, rheumatoid factors, and antinuclear antibodies have all been observed in IM (7). It is possible that the production of lymphocytotoxic antibodies or immune complexes (8) might contribute to the depression of cell-mediated immune responses and the impairment of the autologous MLR. One of the autoantibodies reported to occur in IM has B-lymphocyte specificity (25). Our finding that serum from patients with IM inhibits the autologous, but not the allogeneic, MLR suggests a possible role for serum factors in the impaired autologous MLR in IM. It is possible that anti-B-cell antibodies block the autostimulatory capacity of a subset of B cells which stimulate autologous T-cell proliferation. Alternately, EBV infection of B lymphocytes (26) may alter their autostimulatory capacity.

Summary

The autologous mixed lymphocyte reaction (MLR) is severely impaired in patients with acute infectious mononucleosis. Reactivity returned during the course of convalescence. The allogeneic MLR was not impaired in these patients. B cells from patients with infectious mononucleosis do not stimulate autologous T-cell proliferation, and this observation appears to explain the cellular basis of the impaired autologous MLR in infection. Two explanations for the B-cell defect were considered: (a) the influence of serum factors on B-cell function and (b) the effect of Epstein-Barr virus infection.

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