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Studies of lymphocytotoxins in infectious mononucleosis: reduced lymphocyte killing in the acute phase

J. W. QUIN,* J. A. CHARLESWORTH, C. H. LEE & G. J. MACDONALD *Department of Medicine, Prince Henry Hospital, Sydney, Australia*

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SUMMARY

Fifty sera from twenty-five patients with uncomplicated infectious mononucleosis (IM) were tested for lymphocytotoxic activity (LCA) against pools of lymphocytes collected from (a) normal donors (b) ten patients during the acute phase of IM and (c) four of these patients during the convalescent phase. The LCA of twenty-four sera from patients with systemic lupus erythematosus (SLE) was also tested against the same panels of cells. The percentage of acute phase lymphocytes killed by sera from patients with IM or SLE was significantly less ($P<0{\cdot}001$ and $P<0{\cdot}05$ respectively) than the percentage of normal or convalescent cells killed by the same sera. This reduction could not be explained by lymphocyte donor variation as the same donors were used in the acute and convalescent phases of IM. It is suggested that changes in the surface characteristics of acute IM lymphocytes cause a temporary failure of interaction with circulating lymphocytotoxins. Such a phenomenon could have important influences on the self-limiting nature of the disease.

INTRODUCTION

Lymphocytotoxic activity (LCA) has been demonstrated in the serum of patients with a variety of infectious and immunological disorders, including infectious mononucleosis (IM) and systemic lupus erythematosus (SLE) (Mottironi & Terasaki, 1970; Mittal *et al.*, 1970). In IM, LCA is detectable early in the clinical phase of the disease and may persist for weeks or even months into the convalescent period (Charlesworth *et al.*, 1978). Huang and co-workers (1973) observed that many patients with acute viral infections and high levels of LCA are lymphocytopenic. In contrast, the majority of patients with IM have a lymphocytosis despite the presence of marked LCA and these cells have been demonstrated to be of thymic origin (Sheldon *et al.*, 1973). This observation prompted us to study further lymphocytotoxic activity in uncomplicated IM. The LCA of fifty sera from patients with IM was tested against normal lymphocytes and lymphocytes collected during the acute and convalescent phases of the disease. Twenty-four sera from patients with SLE were analysed similarly as a comparative group. The percentage killing of acute phase IM cells by IM or SLE sera was significantly less than the percentage killing of normal or convalescent cells.

PATIENTS AND METHODS

Patients. Twenty-five patients (thirteen female, twelve male: mean age 23 years, range 16–35 years) with IM, diagnosed by established clinical, haematological and serological criteria (Hoagland, 1972), were studied. Two samples of blood were collected from each patient during the acute in-patient period. A further sample was obtained in the convalescent phase when

* Present Address: Department of Haematology (Blood Bank), Prince Henry Hospital, Sydney, Australia.

Correspondence: Dr J. A. Charlesworth, Department of Medicine, Prince Henry Hospital, Little Bay, 2036, New South Wales, Australia.

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there was evidence of complete serological and clinical recovery. Sera were stored at −30°C until analysed. Thirteen patients with SLE (twelve female, one male: mean age 32 years, range 13–65 years) were also studied.

Isolation of lymphocytes. Normal lymphocytes were obtained, on the day of the assay, from laboratory personnel and healthy volunteers attending a screening survey for serum lipids. Lymphocytes were obtained from at least ten donors on each occasion. Heparinized venous blood was passed through glass columns (1·0 × 20 cm) of nylon wool. The column was then washed with 0·9% saline and the lymphocytes isolated by standard Ficoll–Hypaque density centrifugation (Pharmacia, Sweden). Isolated lymphocytes were washed three times in sterile Hanks' balanced salt solution (Commonwealth Serum Laboratories, Australia) and made up to 10^6 cells/ml.

Lymphocytotoxins. These were quantitated by a modification of the microcytotoxicity method described by Terasaki & McClelland (1964). 100 μl test serum, 100 μl lymphocytes (10^3 cells) and 200 μl rabbit complement were incubated in glass tubes at 15°C for 3 hr. At the end of this period a 200-cell sample was assessed for viability by trypan-blue exclusion. Anti-lymphocyte globulin and a pool of normal human serum (NHS) were included as positive and negative controls in each assay. Mean LCA in twenty-five sera from healthy controls was 2·07 ± 1·77% (mean ± s.d.).

Experimental protocol. Two serum samples were used from each of the twenty-five patients with IM during the acute phase. LCA of these sera was assessed against a pool of normal lymphocytes. In ten of these patients a further 10 ml of heparinized venous blood was taken and the lymphocytes isolated. Morphology was determined on a sequestrene sample taken at the same time. These lymphocytes were included in a second assay for LCA and tested against (a) the patients own serum, (b) 3–6 other IM sera, and (c) 2–3 SLE sera. In this way two serum samples from each IM patient and one sample from each patient with SLE were assessed for LCA against a pool of normal lymphocytes and at least one sample of acute IM lymphocytes. When there was evidence of complete clinical and serological recovery, a second sample of lymphocytes was isolated and the LCA of the same sera, as used in the acute IM lymphocyte assay, retested against the recovered IM lymphocytes. Since most of these sera had been frozen and re-thawed for this latter assay, their LCA was checked against a second pool of normal lymphocytes. In a further experiment lymphocytes were isolated from three patients with SLE and the percentage of these cells killed by sera from three IM patients and seven SLE patients then compared with the percentage of normal lymphocytes killed by the same sera.

Statistics. The statistical difference between the percentage of normal lymphocytes and the percentage of acute or convalescent cells killed by each serum was determined by a paired *t*-test.

RESULTS

Infectious mononucleosis

The LCA of fifty acute IM sera tested against normal lymphocytes and acute IM lymphocytes (37·8 ± 18·6% atypical) is shown in Fig. 1. The mean percentage killing by these sera against normal lymphocytes was 59·86 ± 25·85% (mean ± s.d.). In contrast, the killing of acute IM lymphocytes by the same sera was 29·9 ± 22·34%. This difference in LCA was highly significant ($P < 0.001$). When twenty-six of these acute sera were retested against convalescent lymphocytes from the same donors the percentage killing

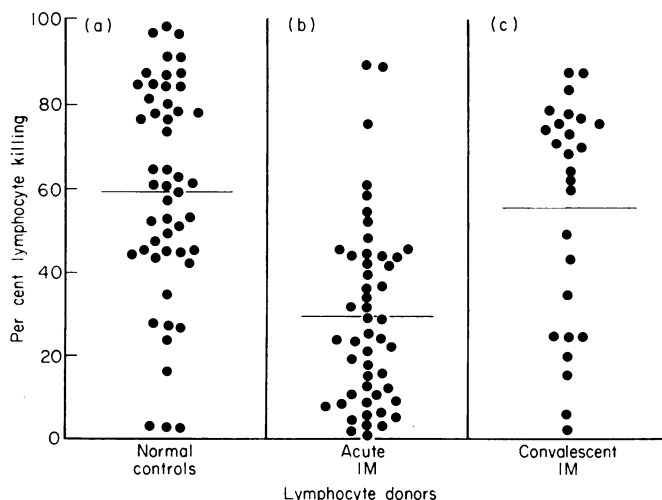

FIG. 1. The LCA of fifty sera from twenty-five patients with IM tested against lymphocytes from: (a) ten healthy donors, (b) ten patients during the acute phase of IM and (c) four patients during the convalescent phase of IM.

was not different from that observed against normal lymphocytes ($P>0.05$) (see Fig. 1). Two of these acute sera were tested against acute and convalescent cells from three different donors. With each donor the LCA of the sera tested against acute lymphocytes was significantly less ($P<0.05$) than the LCA of the same sera tested against convalescent cells.

The mean LCA of twelve acute IM sera tested against two different pools of normal lymphocytes (ten donors in each pool) was $57.5\pm28.4\%$ and $63.8\pm29.7\%$. This difference in LCA was not significant.

In addition the LCA of three acute IM sera was tested against lymphocytes isolated from patients with SLE. The percentage killing of these cells was comparable to the killing of normal cells by the same three IM sera.

Systemic lupus erythematosus

The results for LCA in twenty-four sera tested against normal lymphocytes and acute IM lymphocytes are shown in Fig. 2. The mean percentage killing for these sera against normal lymphocytes was

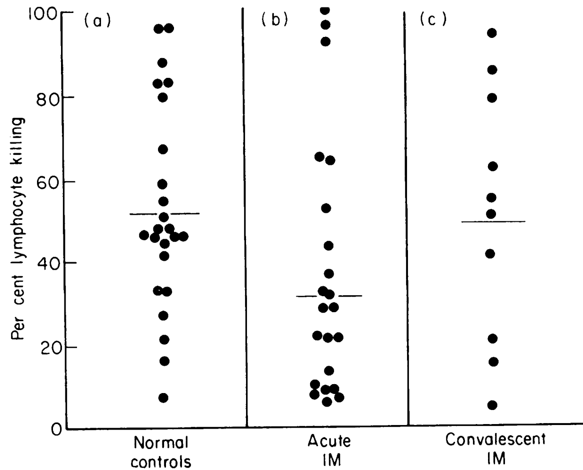

FIG. 2. The LCA of twenty-four sera from 13 patients with SLE tested against lymphocytes from: (a) ten healthy donors, (b) six patients during the acute phase of IM and (c) three patients during the convalescent phase of IM.

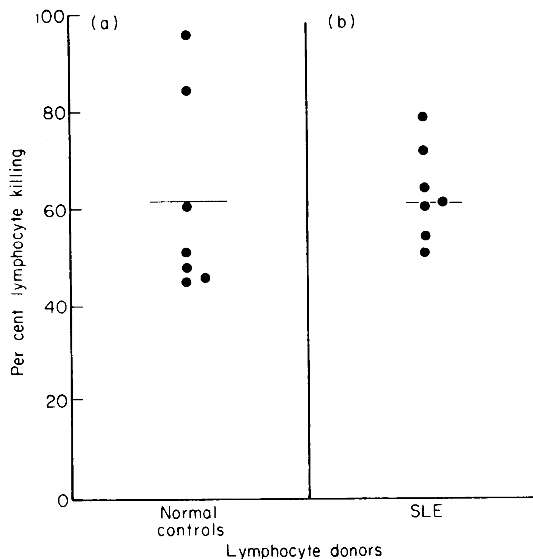

FIG. 3. The LCA of seven sera from seven patients with SLE tested against lymphocytes from: (a) ten healthy donors and (b) three patients with SLE.

52·75±24·72% and against acute IM lymphocytes 34·08±29·69%. This difference was significant ($P<0·05$). In contrast when ten of these sera were tested against convalescent IM lymphocytes from the same donors, the percentage killing was not significantly different from that observed against normal lymphocytes.

The LCA for seven SLE sera tested against normal lymphocytes and SLE lymphocytes is shown in Fig. 3. There was no significant difference between the percentage of normal and diseased lymphocytes killed by these sera.

The mean LCA of ten sera from patients with SLE tested against two different pools of normal lymphocytes was 52·6±23·6% and 46·1±29·9%. This difference in LCA was not significant.

DISCUSSION

This study demonstrates the relative failure of sera from patients with IM or SLE to kill peripheral lymphocytes obtained from subjects during the acute phase of IM. We have shown LCA in IM to be directed against T lymphocytes (Charlesworth *et al.*, 1978) and the temporary loss of this activity during the acute phase seems likely to result from an alteration in the surface characteristics of these cells. Although it is established that the Epstein–Barr virus (EBV) specifically infects B lymphocytes (Jondal & Klein, 1973), gross morphological changes are observed in T lymphocytes (McKenna *et al.*, 1977) and these changes are thought to represent host reaction to the EBV infected cells (Svedmyr & Jondal, 1975). In our patients, 37·8±18·6% of acute phase peripheral lymphocytes were atypical.

Moretta, Mingari & Romanzi (1978) have reported the disappearance of IgG-Fc receptors from T cells following their interaction with IgG-containing immune complexes. This loss of surface receptors in response to circulating ligand—whether this be antibody or immune complex—is a well recognized phenomenon (Taylor *et al.*, 1971; de Petris & Raff, 1972). The acute phase of IM is characterized by the formation of IgM-containing complexes (Quin, Charlesworth & Macdonald, 1979) and serum lymphocytotoxins present during this stage are also known to involve IgM (Mottironi & Terasaki, 1970). Recent work demonstrates significant loss of IgM-Fc receptors from the surface of T lymphocytes during the acute phase of this disease (Haynes *et al.*, 1979). It seems plausible that, as with IgG-mediated systems, these circulating lymphocytotoxins lead to loss of specific surface receptors and impaired T cell killing. We have shown this lymphocytotoxicity to result at least in part from antigen–antibody complex activity (Quin *et al.*, 1980) rather than antibody-mediated cytotoxicity.

The role of these phenomena in limiting the duration of EBV-induced lymphoproliferation remains to be investigated. In mouse thymus-leukaemia it is considered that loss of surface receptors leads to the escape of leukaemic cells from the host immune response. It has also been shown that removal of antibody from cell culture permits the re-synthesis of receptors within 6–24 hr (Loor, Forni & Pernis, 1972). Clearly, the duration of the proposed defect in receptor regulation in the acute phase of IM could be crucial to the evolution of EBV in man.

REFERENCES

Charlesworth, J.A., Quin, J.W., Macdonald, G.J., Lennane, R.J. & Boughton, C.R. (1978) Complement, lymphocytotoxins and immune complexes in infectious mononucleosis: serial studies in uncomplicated cases. *Clin. exp. Immunol.* **34**, 241.

De Petris, S. & Raff, M.C. (1972) Distribution of immunoglobulin on the surface of mouse lymphoid cells as determined by immunoferritin electron microscopy. Antibody-induced, temperature-dependent redistribution and its implications for membrane structure. *Eur. J. Immunol.* **2**, 523.

Haynes, B.F., Schooley, R.M., Grouse, J.E., Payling Wright, C.R., Dolin, R. & Fauci, A.S. (1979) Characterization of thymus-derived lymphocyte subsets in acute Epstein–Barr virus-induced infectious mononucleosis. *J. Immunol.* **122**, 699.

Hoagland, R.J. (1972) *Infectious mononucleosis* (ed. by P. R. Glade), p. 1, J. B. Lippincott & Co., Philadelphia.

Huang, S.W., Lattos, D.B., Nelson, D.B., Reeb, K. & Hong, R. (1973) Antibody-associated lymphotoxin in acute infection. *J. clin. Invest.* **52**, 1033.

Jondal, M. & Klein, G. (1973) Surface markers on human B and T lymphocytes. II. Presence of Epstein–Barr virus receptors on B lymphocytes. *J. exp. Med.* **138**, 1365.

Loor, F., Forni L. & Pernis, B. (1972) The dynamic state of the lymphocyte membrane. Factors affecting the distribution and turnover of surface immunoglobulins. *Eur. J. Immunol.* **2**, 203.

McKenna, R.W., Parkin, J., Gajl-Peczalska, K.J., Kersey, J.H. & Brunning, R.D. (1977) Ultrastructural, cytochemical and membrane surface marker characteristics of the atypical lymphocytes in infectious mononucleosis. *Blood*, **50**, 505.

Mittal, K.K., Rossen, R.D., Sharp, J.T., Lidsky, M.D. & Butler, W.T. (1970) Lymphocyte cytotoxic antibodies in systemic lupus erythematosus. *Nature*, **225**, 1255.

Moretta, L., Mingari, M.C. & Romanzi, C.A. (1978) Loss of Fc receptors for IgG from human T lymphocytes exposed to IgG immune complexes. *Nature*, **272**, 618.

Mottironi, V.D. & Terasaki, P.I. (1970) Lymphocytotoxins in disease. I. Infectious mononucleosis, rubella and measles. In *Histocompatibility Testing* (ed. by P. I. Terasaki), p. 301. Williams & Wilkins Co., Baltimore.

Quin, J.W., Charlesworth, J.A., Bowman, C. & MacDonald, G.J. (1980) Studies of lymphocytotoxins in infectious mononucleosis and systematic lupus erythematosus: evidence for immune complex-mediated cytotoxicity. *Clin. exp. Immunol.* **39**, 593.

Quin, J.W., Charlesworth, J.A. & Macdonald, G.J. (1979) The detection of immune complexes of different immunoglobulin class. *Aust. NZ. J. Med.* (In press.)

Sheldon, P.J., Papamichail, M., Hemsted, E.H. & Holborow, E.J. (1973) Thymic origin of atypical lymphoid cells in infectious mononucleosis. *Lancet*, **i**, 1153.

Svedmyr, E. & Jondal, M. (1975) Cytotoxic effector cells specific for B cell lines transferred by Epstein–Barr virus are present in patients with infectious mononucleosis. *Proc. Natl. Acad. Sci. USA.* **72**, 1622.

Taylor, R.B., Duffus, W.P.H., Raff, M.C. & De Petris, S. (1971) Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nature: New Biol.* **233**, 225.

Terasaki, P.I. & McClelland, J.D. (1964) Microdroplet assay of human serum cytotoxins. *Nature*, **204**, 998.