Increased Frequency of Lymphocytotoxic Antibodies in Patients with Multiple Sclerosis Correlated with Disease Activity

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Increased Frequency of Lymphocytotoxic Antibodies in Patients with Multiple Sclerosis Correlated with Disease Activity. Tohoku J. exp. Med., 1982, 136 (2), 121–128 — Cold-reactive lymphocytotoxic antibodies (LCA) were investigated in sera from patients with multiple sclerosis (MS) in relation to disease activity. When a serum with more than 20% cytotoxicity is considered positive for the presence of LCA, the positive frequency at remission was 21% (6/28), but at exacerbation it was 83% (10/12). All the sera from normal controls were negative. The average of cytotoxicity expressed as a % killing of target cells by sera at remission was 13%; on the other hand, at exacerbation it was 39%. These results showed that in multiple sclerosis the level of LCA was not persistently high, but increased correlating with the disease activity. From these findings and previous experiments of rescue of measles virus antigens, it was suggested that multiple sclerosis is likely a virus-induced autoimmune disease.

Cold-reactive lymphocytotoxic antibodies (LCA) have been detected in sera from patients with a variety of autoimmune diseases (Terasaki et al. 1970; Mittal et al. 1970; Kuwert and Bertrams 1972; Korsmeyer et al. 1974; Strickland et al. 1975; Schocket et al. 1977; Weiner and Schocket 1979). LCA are regarded as autoantibodies (Messner et al. 1975) which are directed to the surface antigens of normal lymphocytes and the appearance of LCA has been suggested to be related to a viral infection (Huang et al. 1973; DeHoratius and Messner 1975; Folomeeva et al. 1978; Schocket and Weiner 1978). The frequency of LCA in sera from patients with multiple sclerosis (MS) was 34% reported by Kuwert and Bertrams (1972) and 46% and 67% reported by Schocket et al. (1977), but the correlation between LCA levels and disease activity has not been evaluated.

On the other hand, following the report of Prasad et al. (1977), we also rescued measles virus antigens from the jejunum of 7 out of 11 MS patients by the cell fusion technique using polyethylene glycol and proposed a hypothesis for
the mechanism of demyelination in the central nervous system of MS patients (Ebina et al. 1979).

The purpose of this paper is to confirm our hypothesis and to describe the relationship between LCA level in sera from patients with MS and its disease activity.

MATERIALS AND METHODS

Sera. Double serum samples were collected from 20 patients (8 males and 12 females) with definite MS as defined by the Schumacher Committee criteria (Schumacher et al. 1965). Among 40 serum specimens, 28 were obtained at a clinically stable stage and the rest 12 at the time of exacerbation. Age (19 to 66) and sex matched control sera were obtained from 20 normal persons. Serum from a patient with systemic lupus erythematosus (SLE) was served as the positive control.

Target cells. Target cells used in the assay were lymphocytes prepared by centrifugation on a Ficoll-Isopaque solution (Pharmacia Fine Chemicals, Uppsala, Sweden) from normal human heparinized blood. The test sera were always assayed in twofold diluted and tested against two healthy donors' cells.

Lymphocytotoxic antibodies. LCA were tested using Falcon #3040 microplate. Twenty-five μl of 4×10⁶ target cells/ml in RPMI 1640 medium was first incubated in a well of a microplate with 25 μl of test serum at 15°C for 30 min. Twenty-five μl of normal rabbit serum was then added to the well as complement and the mixture was incubated at 15°C for 3 hr. During the incubation the microplate was shaken by a micro-mixer for 15 sec every 30 min. One drop of 0.8% trypan-blue solution was added to the well and a percentage of killed cells was determined by a trypan-blue exclusion test, counting over two hundred cells microscopically on a hemocytometer. The medium of RPMI 1640 supplemented with 5% heated normal human serum was used for the dilution of test serum in order to reduce nonspecific cytotoxicity.

Fractionation of lymphocytes by nylon wool column. Lymphocytes suspended in warmed medium at 37°C of RPMI 1640 added with 10% fetal calf serum were applied to a nylon wool column and incubated at 37°C for 1 hr. After passing through the nylon wool column the lymphocytes were washed three times by RPMI 1640 and examined as target cells against sera from five MS patients and one normal control.

Anti-human γ serum and anti-human μ serum. Sera obtained from rabbits immunized with purified human IgG or IgM were further absorbed with appropriate immunoabsorbants and used as monospecific antisera for human IgG or IgM.

The mixture of one volume of test serum and one volume of serial diluted rabbit anti-IgG or anti-IgM serum was incubated at 37°C for 90 min. The target cells were then added to the mixture and the cytotoxicity was measured. This experiment was performed on five sera with increased cytotoxicity from MS patients in order to determine the immunoglobulin class of LCA. The same serum incubated with heated normal rabbit serum was also tested as a control.

RESULTS

Lymphocytotoxic antibodies in sera from patients with multiple sclerosis

The averages of cytotoxicity in 20 samples from normal controls and 28 samples from MS patients in a clinically stable state were 7.7% (5.2–10.3%, p=0.05) and 13.0% (9.7–16.3%, p=0.05), respectively. On the other hand, the average of cytotoxicity in 12 samples from MS patients with exacerbation was 39.2% (28.2–50.2%, p=0.05) and was significantly greater than the average of MS patients in a clinically stable state (Fig. 1). From these results, LCA were shown to be
Lymphocytotoxic Antibodies in MS

increased at the period of clinical exacerbation. When a serum with a cytotoxicity above 20% is considered positive for LCA, 10 out of 12 samples from MS patients at exacerbation (83%) and 6 out of 28 samples from MS patients at remission (21%) were positive, but all of the normal controls were negative.

Characterization of lymphocytotoxic antibodies

The positive cytotoxicity became negative when rabbit serum was not added or it was inactivated at 56°C for 30 min before adding. Therefore, this cytotoxicity was shown to be complement-dependent one.

The optimum temperature and necessary incubation time for the assay of LCA were found to be 15°C and 3 hr among the results at 4°C, 15°C and 37°C for 3 hr and at 15°C for 1, 2 and 3 hr.

The positive serum was able to be diluted even to 128-fold (Fig. 2).

Fig. 1. Cytotoxicities in MS sera at remission and at exacerbation and in control sera. The horizontal line represents the average cytotoxicity of each group.

Fig. 2. Effect of serial dilution of sera from three MS patients (1 and 2 were at exacerbation and 3 was at remission) and one normal control of cytotoxicity. ○=Complement control.
Next, in order to ascertain which lymphocytes are target cells, the whole lymphocytes and the lymphocytes consisting of almost T cells after passing through the nylon wool column were studied as the target cells at the same time. As no difference was found in cytotoxicity between whole lymphocytes and T cell rich fraction, it is suggested that the target cells which LCA are directed may be mainly T cells (Fig. 3).

Several positive sera were incubated with serially diluted rabbit anti-human IgG or IgM sera. In all cases the positive cytotoxicity was completely reduced to negative after incubation with undiluted anti-\(\mu\) rabbit serum, but in all but a case of N.M. the cytotoxicity remained enough after incubation with anti-\(\gamma\) rabbit serum (Fig. 4). These results showed that LCA belong chiefly to IgM, but in some cases partly to IgG.

Fig. 3. Cytotoxicity against the lymphocytes before or after passing through the nylon wool column. □=before, ■=after.

Fig. 4. Cytotoxicities in MS sera after incubation with antihuman IgG or IgM rabbit serum or with normal rabbit serum (NRS).
Lymphocytotoxic antibodies (LCA) were studied on sera from MS patients and normal controls by counting the number of the killed cells microscopically using a modified microdroplet method of Terasaki and McClelland (1964). Cytotoxicity was determined as a % killing of target cells by test serum and when a serum with a cytotoxicity above 20% is regarded positive for LCA, the frequency of LCA was 0% (0/20) in normal controls and 40% (16/40) in all the cases of MS. The figure of 40% positives in MS is almost consistent with the figure of 34% reported by Kuwert and Bertrams (1972) and 56% by Schocket et al. (1977). But comparing the frequency of LCA in sera obtained at exacerbation with that of LCA in sera obtained at remission, it was shown that the frequency at exacerbation was 83% (10/12), which was greatly higher than the frequency at remission of 21% (6/28). Furthermore, mean % cytotoxicity at exacerbation (39%) was also much higher than that at remission (13%). Although the frequency of LCA in normal controls is 12.5% in the report by Kuwert and Bertrams (1972), 8% and 6% in the reports by Schocket et al. (1977) and Schocket and Weiner (1978), no positive sera were found in normal controls in our experiments. This was probably due to the supplement of 1.25% normal human serum to the assay system, which might be able to prevent the cells from dying nonspecifically during the incubation.

The LCA shown here are complement dependent antibodies, react optimally at 15°C for 3 hr, chiefly belong to IgM antibodies and react mainly against T cells. The positive serum was able to be diluted 128-fold. These characters of LCA in MS are similar to those in SLE (Terasaki et al. 1970; Lies et al. 1973; Michlmayr et al. 1976), but the natures of LCA found in sera from patients with SLE are not always consistent among several reports (Winfield et al. 1975; Searles et al. 1977).

The presence of LCA has been reported in sera from patients with a variety of autoimmune diseases, including SLE, rheumatoid arthritis, MS and inflammatory bowel disease. The LCA also appeared temporarily in sera from patients with acute viral infections (Huang et al. 1973) and from normal subjects after treatment with viral vaccines (Kreisler et al. 1970). But, although the assay of LCA is all dependent on the phenomenon of killing normal lymphocytes, all the LCA demonstrated in the above mentioned disease may not be the same (Winfield et al. 1975). The LCA which react enough at 37°C are reported (Mittal et al. 1970; Huang et al. 1973) and the LCA found in sera from SLE patients either can be absorbed or cannot be absorbed with brain tissue (Bluestein and Zvaifler 1976; Bresnihan et al. 1977; Winfield et al. 1978). The antigens on the surface of lymphocyte to which LCA may be directed are not yet known.

Anyway the presence of LCA has been demonstrated frequently in both viral diseases and the diseases that are suspected to be related to viral infections. Schocket and Weiner (1978) also found that lymphocytotoxic antibodies were specifically present in sera from patients with MS and that a significantly greater frequency of these antibodies could also be demonstrated in the patients'
household members compared with siblings living elsewhere or healthy controls. They considered from these results that the increased frequency of LCA in MS patients and their family members is an indicator of the presence of a transmissible agent, probably a virus, in many patients with MS, and further that continued exposure to the inducing agent is necessary for maintenance of detectable antibody levels. However, our results showed that the level of LCA is not persistently increased in MS patients, but rather becomes increased correlating with disease activity. Therefore, the patients with MS could be infected latently by an infectious agent, if any, in the body rather than are continuously exposed to it and the causative agent and mechanism could be worked out of the brain (Weiner and Schocket 1979).

We recently rescued measles virus antigens from the jejunum of 7 out of 11 MS patients in a clinically stable state by the cell fusion technique (Ebina et al. 1979) and hypothesized that the manifestation of measles virus in jejunal cells at certain time, probably at exacerbation, by an unknown mechanism results in the infection of the nascent T lymphocytes and the modification of the T cell membranes. Then the modified T lymphocytes can be recognized as foreign bodies and anti-modified T lymphocytes sera will be produced as autoantibodies, which are now proved to be detectable as lymphocytotoxic antibodies frequently in sera from MS patients at clinical exacerbation. As a cell membrane of T lymphocyte shares common antigens with human brain tissue (Bluestein and Zvaifler 1976; Bresnihan et al. 1977; Winfield et al. 1978), it is likely that the virus induced lymphocytotoxic antibodies would further cross-react with brain antigens, leading to the immunopathologic demyelination either directly or by an immune “by-stander” effect (Lipton and DalCanto 1976; DalCanto et al. 1979) in the central nervous system of multiple sclerosis.

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Lymphocytotoxic Antibodies in MS 127


