ANTIBODIES TO A SPECIFIC SURFACE ANTIGEN OF T CELLS IN HUMAN SERA INHIBITING MIXED LEUKOCYTE CULTURE REACTIONS*

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The mixed leukocyte culture (MLC) has been used for a number of years as a model for the recognition aspect of the homograft reaction. There appears to be a significant correlation between skin graft survival and MLC reactivity (1). Newer evidence in man and mouse has indicated that the major histocompatibility loci may not be primarily involved in the stimulation phase but that loci associated with immune response (Ir) gene differences are more significant (2, 3). Recently Eijssenvoogel et al. demonstrated a separate inheritance of the HL-A antigens and the determinants eliciting pronounced MLC, probably by recombination, which implied independence of this MLC system from both the LA and FOUR loci (4).

A number of different human sera have been found that strongly inhibit the MLC reaction (5–7). This has been particularly evident for sera from patients with systemic lupus erythematosus (SLE) where evidence has been obtained that antibodies inhibiting the responder cells in the MLC reaction are involved (8). Cytotoxic antibodies to T cells have also been described in the sera of patients with SLE (9). The present studies were undertaken in an attempt to define the specificity of the antibodies involved in the MLC suppression. Membrane antigens reacting with these antibodies were investigated by the method of lymphocyte surface radioiodination (10). One antigen on T cells defined by this procedure appeared particularly relevant.

**Materials and Methods**

The method used for the unidirectional mixed leukocyte culture (MLC) has been performed in a microsystem as described by Hartzman et al. (11).

Absorptions of normal and SLE sera were carried out routinely at two temperatures. 4 ml of heat-inactivated serum was incubated with equal volume of packed ABO red cells at 4°C, and then the supernatant with another 4 ml of packed cells at 37°C. In addition, certain SLE sera were absorbed further in the same fashion with isolated and pooled lymphocytes from patients with chronic lymphocytic leukemia (CLL) having high peripheral blood counts; then with packed platelets pooled from ten different donors; and with allogeneic kidney cells. Beyond that a few sera were absorbed with allogeneic thymus or spleen cells or with buffy coat cells.

For the enzymatic surface radioiodination (SRI) a modification of the method described by Baur et al. (10) using lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.) to radioiodinate the lymphocyte membranes was applied. For this procedure the lymphocyte populations were first kept in short-term cultures, which resulted in shedding of the autologous antibodies coated on the cells in vivo without loss of viability (8). The cells were then purified further by passage through a nylon-wool column as a second and through Sephadex G-10 columns as a third purification step to remove phagocytic cells.

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The radioiodinated lymphocytes were solubilized with the nonionic detergent NP-40 (Shell Oil Company, New York). In a first step with rabbit antihuman Ig and subsequent goat anti-rabbit Ig antibodies in excess, all lymphocyte-derived immunoglobulin molecules were precipitated out by this sandwich technique. The remaining supernatant was incubated first with a normal or SLE serum or its IgG fraction and subsequently with the goat antihuman Ig antisera. The formed specific precipitate was solubilized and run on a 5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS). The radioactive bands obtained represent antigen targets on the lymphocyte surface identified by specific antibody present in the lupus sera. Radiolabeled marker proteins were: IgG (~150,000 daltons), γ-chains (~50,000 daltons), light chains (L~24,000 daltons), and β2-microglobulin (B2M~11,500 daltons).

RESULTS

Numerous experiments were carried out where the response of different lymphocytes to stimulator cells in MLC reactions was compared in the presence of normal vs. SLE serum. Marked suppression was noted in certain SLE sera. This was maximal when the SLE cells were used as responders in the presence of the corresponding sera. Fig. 1 illustrates one series of representative experiments.

By testing 34 SLE patients in a similar fashion it was found that 19 out of these showed an impaired MLC reactivity and 7 out of the 19 gave a complete inhibition of the MLC. The SLE sera involved primarily in this suppression of cell-mediated immunity were also able to block a number of allogeneic lymphocyte combinations of normal persons. In comparing the blocking capacity and

![Graph](image)

**Fig. 1.** Reactivity and kinetics of unidirectional MLC reactions all triggered by the same stimulating lymphocyte population A. Upper curve (O—O) represents that for normal responder lymphocytes, N, in normal AB serum; middle curve (●—●) is that for SLE responder cells, Go, in normal AB serum; and lower curve (◆—◆) is that for SLE responder cells, GO, in SLE serum GO. Complete suppression is noted in the latter case.
the cytotoxic potential of the lupus sera tested, a discrepancy between these two functions became apparent in vitro with some sera showing a strong MLC suppressive but no lymphocytotoxic effect. Considerable evidence was obtained by gel filtration of the SLE sera on Sephadex G-200 and by Pevikon block electrophoresis, indicating that the main blocking activity in the MLC reaction was due to antibodies of the IgG class.

Fig. 2 A illustrates the lymphocyte surface profile obtained from the peripheral blood lymphocytes of SLE patient Har on SDS acrylamide electrophoresis.
using the autologous serum as the reactive antiserum. Four different targets can be identified as separate peaks with the SLE serum Har that do not appear with the control serum that was nonsuppressive in the MLC system. From these experiments and others where normal peripheral blood lymphocytes were used as targets, the impression was gained that active SLE sera contain a whole group of different antibodies directed against different lymphocyte surface structures. Certain SLE sera were thoroughly absorbed with B cells, platelets, etc. to remove HL-A and other antibodies as described under Materials and Methods. The absorbed sera were still strongly suppressive in the MLC when the autologous lymphocytes were used as the responder population. Direct evidence against involvement of HL-A antibodies was shown by the continued blocking effect of absorbed SLE serum Har using an HL-A identical but MLC reacting lymphocyte population from an unrelated donor as stimulator cells. Fig. 2 B shows that this absorbed serum localized primarily one labeled surface structure at about Fraction number 50. As can be calculated from the approximative position of included marker proteins this peak corresponds to a molecular weight (mol wt) of about 15,000 daltons. With the exception of a minor peak at Fraction 25 all the other antibody activities were absorbed out. The same

![Diagram](image-url)

**Fig. 3.** SDS polyacrylamide gel electrophoresis pattern of human thymocytes (A) and a CLL "B cell" population (B) using unabsorbed SLE serum Har (○—○) and normal AB serum (×—×). Marker positions are included as in Fig. 2. In A only the small component at Fraction 50 is again evident, whereas this constituent is missing on "B lymphocytes" (B).
surface iodination pattern was obtained when allogeneic thymocytes were used together with the unabsorbed serum Har (Fig. 3 A); this was not seen for B cells (Fig. 3 B).

Two other SLE sera that showed strong inhibition of MLC gave similar results with specific reactivity for the low mol wt component. This was not observed for a limited number of sera that failed to inhibit the MLC reaction.

DISCUSSION

It appears from the above experiments that T cells and thymocytes possess a low mol wt surface constituent to which antibodies are sometimes found in human sera. Although most of the work was carried out on SLE sera, similar antibodies were found in sera from certain individuals with other conditions and in certain allogeneic antisera. All the sera showing reactivity against this material also blocked strongly the responder cells in the MLC reaction; various control sera that did not block the MLC failed to show such specificity. It should be emphasized, however, that further sera need to be studied to establish firmly this apparent association. Absorption of these antisera with B cells, platelets, and a variety of other cells failed to remove either the MLC-blocking effect or the reactivity for the low mol wt surface constituent. Absorption with thymocytes or spleen cells removed both effects.

A number of the absorbed sera that showed MLC inhibition also showed a small higher mol wt component which may be significant. The possibility also remains that the blocking antibodies react with a constituent that is not solubilized by the ionic detergent utilized. Another possibility is that the antibodies that block the MLC reaction, even if they are directed to the low mol wt component, are blocking because of reactivity to a site close to or adjoining the receptor site. At any rate, irrespective of these various possibilities, the low mol wt antigen clearly represents a new surface marker of T cells to which autoantibodies are frequently produced.

Current efforts are directed toward the preparative isolation of the low mol wt component so that its effect on the blocking antibodies can be determined directly. If this is successful, it appears possible that the low mol wt component participates in the recognitive phase involved in the MLC reaction. Recently antisera have been produced in mice that react with an antigen on a subpopulation of T cells (12) that appears related to the Ir gene product postulated by Benacerraf and McDevitt (13).

SUMMARY

Surface radioiodination of lymphocytes by the lactoperoxidase procedure has permitted demonstration of an assortment of different antibodies to lymphocytes in the sera of patients with systemic lupus erythematosus. One type of antibody proved of special interest because it appeared to be associated with inhibition of the responder cells in the mixed leukocyte culture reactions. This reacted with an antigen on T cells and thymocytes which on sodium dodecyl
sulfate acrylamide gel electrophoresis showed a molecular weight of approximately 15,000 daltons. The possible relation of this antigen to T cell receptors and products of immune response genes is discussed.

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