*Brief Definitive Report*

# Autoantibodies Specific for Different Isoforms of CD45 in Systemic Lupus Erythematosus

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## Summary

Nearly one-third of IgM antilymphocyte autoantibody-positive sera from patients with systemic lupus erythematosus (SLE) contain IgM antibodies to one or more 180–220-kD molecules (p180, p190, p205, and p220) in blots of glycoproteins purified from T cells by wheat germ agglutinin affinity chromatography. Identity of these IgM targets with multiple isoforms of CD45 was established by their specific depletion from T cell glycoproteins by immunoprecipitation with T191, a monoclonal antibody (mAb) that reacts with an epitope common to all CD45 isoforms. Although the anti-CD45 autoantibodies recognize higher molecular weight isoforms primarily, antigenic specificity in this system is quite heterogeneous and includes multiple distinct CD45 isoforms on different types of T cells that are, at least in part, different from those reactive with mAbs 2H4 and UCHL-1. Because CD45 is a major membrane protein tyrosine phosphatase that plays a critical role in antigen-induced T cell activation, the present data may be relevant to some of the antilymphocyte antibody-mediated immunologic abnormalities that characterize SLE and related autoimmune diseases.

Study of the cell-type specificity of IgM antilymphocyte autoantibodies (cold lymphocytotoxins) in SLE has established an overall preferential reactivity with peripheral $CD4^+$ T cells expressing high levels of CD45RA isoforms defined by monoclonal anti-2H4 (suppressor-inducer phenotype) (1, 2) and with activated T cells (3). Observations in this system further suggest that such autoantibodies deplete circulating $CD4^+/CD45RA^+$ T cells in patients with active disease and inhibit antigen-induced T cell proliferation and suppressor-inducer function in polyclonal B cell activation in vitro (2–4). Collectively, the available data argue that autoantibodies to lymphocytes contribute substantially to the complex immunoregulatory abnormalities that characterize patients with SLE and related systemic autoimmune disorders (5, 6). Progress in this area has been limited, however, by the paucity of unambiguous information concerning the molecular nature of the target antigens involved. In the present investigation, several ~200-kD glycoproteins shown previously to be IgM antilymphocyte autoantibody targets in this disorder (7) were identified as isoforms of the CD45 molecular complex (leukocyte common antigen). Because different isoforms of CD45 define functionally important lymphocyte subpopulations and are pivotal in T cell regulation of cell signaling during the immune response via their protein tyrosine phosphatase activity (8–10), these data may be relevant to basic mechanisms underlying immunologic dysfunction in SLE and other autoimmune diseases.

## Materials and Methods

*Patients and Serum.* Venous blood was obtained from 43 patients attending the University of North Carolina Lupus Clinic who met the revised criteria of the American College of Rheumatology for classification as SLE (11), and from 10 normal subjects. After separation from blood, serum was aliquoted and stored at −70°C. Each serum aliquot was heated at 56°C for 1 h immediately before use to inactivate complement.

*Cells.* E6-1, a $CD4^+/CD45RA^+/TCR\text{-}\alpha/\beta^+$ Jurkat T cell line phenotypically similar to resting peripheral lymphocytes, and PEER, a $CD4^-/CD8^-/CD45RO^+/TCR\text{-}\alpha/\beta^+$ cell line, were cultured in RPMI 1640 medium containing 10% FCS, glutamine, and antibiotics.

*Special Immunological Reagents.* T191, an IgG2a mAb to conventional CD45 (12) was provided by Dr. R. Mittler, Bristol-Meyers Co., Wallingford, CT. Anti-2H4, a mAb to CD45RA (13), was a gift from Dr. C. Morimoto, Harvard Medical School, Boston, MA. UCHL-1, a mAb to CD45RO (14), was purchased from Dako Corp., Santa Barbara, CA.

*Indirect Immunofluorescence and Flow Cytometry.* Binding of mAbs and SLE IgM to the T cell lines was detected by indirect immunofluorescence at 4°C using FITC-conjugated $F(ab')_2$ goat anti-mouse IgG or anti-human IgM (Cappel Laboratories, Cochranville, PA) (1).

*Lectin Affinity Chromatography.* Glycoproteins from E6-1 and PEER were purified from 0.5% Renex 30 detergent cell lysates by elution from agarose-conjugated wheat germ agglutinin (WGA) (Vector Laboratories, Burlingame, CA) with 10% *N*-acetylglucosamine (7).

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*Preclearance Experiments.* All isoforms of CD45 were pre-cleared from E6-1 and PEER WGA eluates by immunoprecipitation with T191 and protein A-Sepharose (Zymed Laboratories, San Francisco, CA), as described in detail previously (15). Control immunoprecipitations were performed with UPC10, a monoclonal IgG2a. SLE patient sera were depleted of antilymphocyte antibodies by incubation with viable E6-1 cells, as described previously (7).

*Electrophoresis and Immunoblotting.* Detergent cell lysates, lectin eluates, or ~32-fold enriched plasma membranes (16) in Laemmli sample buffer were subjected to electrophoresis on 6 or 8% SDS-polyacrylamide slab gels under reducing conditions, and electro-eluted to nitrocellulose. The blots were probed with appropriate dilutions of SLE patient serum or normal human serum and stained with biotinylated anti-human IgM or IgG antibodies (Tago Inc., Burlingame, CA) and avidin-biotinylated horseradish peroxidase (Vectastain ABC kit; Vector Laboratories, Inc., Burlingame, CA), as described in detail previously (7, 15).

## Results and Discussion

Nearly one-third (10/29) of SLE patient sera positive for IgM antilymphocyte antibodies against E6-1 in indirect immunofluorescence assays contained IgM that stained one or more ~200-kD proteins (gp ~200) on blots of E6-1 plasma membranes or WGA-purified glycoproteins (Fig. 1 *a*). IgM antibodies to gp ~200 were not demonstrable in 10 control sera from normal subjects or in 14 antilymphocyte autoantibody-negative SLE sera. When blots of glycoproteins from E6-1 and a second T cell line, PEER, were separated on 6% SDS-polyacrylamide gels using extended electrophoresis times, gp ~200 was resolved into four proteins of $M_r$ 180–220,000. As illustrated for representative SLE patients in Fig. 1 *b*, anti-gp ~200–positive sera stained proteins of 190, 205, and/or 220 kD with E6-1 glycoproteins (lanes *1–3*, *6*), but, with one exception, did not react with PEER glycoproteins (e.g., compare staining of E6-1 and PEER by St serum in lanes *6* and 7, respectively). The exceptional serum (patient Tu) contained IgM that stained several E6-1 proteins (lane *8*) and a 180-kD protein with PEER (lane *9*). The general absence of anti-gp ~200 reactivity with PEER glycoproteins in the panel of SLE sera studied probably explains the dramatically lower IgM indirect immunofluorescent staining of PEER (32 ± 19% IgM-stained cells) relative to E6-1 (62 ± 28% IgM-stained cells).

The similarity between the molecular mass of the four SLE IgM antibody targets and that of the p220/p205/p190/p180 isoforms of CD45 known to be expressed on different populations of lymphocytes (17) suggested that the SLE IgM antibodies were directed against individual isoforms of CD45 expressed differently on E6-1 and PEER. In indirect immunofluorescence experiments to test this possibility, E6-1 was stained by T191, a mAb that recognizes all CD45 isoforms, and by anti-2H4 (CD45RA specific), but not by UCHL-1 (CD45RO specific); conversely, PEER reacted with T191 and UCHL-1, but not with anti-2H4. Taken together, these data strongly suggested that the IgM anti-gp ~200 antibodies in the SLE sera recognize specific isoforms of CD45.

The identity of gp ~200 with CD45 was established by depleting CD45 from WGA eluates of E6-1 and PEER with T191. Immunoblots of CD45-depleted glycoproteins no longer were stained by SLE IgM (Fig. 2 *a*). These data, together with the results of the immunofluorescence experiments and the informative staining patterns of Tu IgM for E6-1 and PEER glycoproteins, indicate unequivocally that SLE IgM recognized isoform-specific determinants of CD45 expressed on the cell surface, rather than the intracytoplasmic domain of

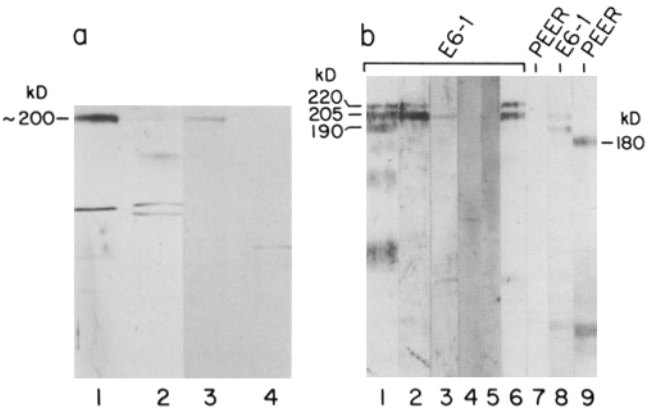


**Figure 1.** IgM antibodies in SLE patient sera recognize different ~200-kD T cell glycoproteins. (*a*) Equal concentrations of protein in enriched plasma membranes (lane *1*), whole cell lysate (lanes *2* and *4*), or glycoproteins purified from E6-1 cells by WGA affinity chromatography (lane *3*) were separated on 8% SDS-polyacrylamide gels, blotted to nitrocellulose, and probed with IgM from SLE patients St (lanes *1* and *2*) and Wa (lanes *3* and *4*), as described in Materials and Methods. (*b*) Blots were prepared with E6-1 or PEER WGA eluates separated on 6% SDS-polyacrylamide gels using prolonged electrophoresis times, and probed with antilymphocyte antibody-positive SLE sera (St, lanes *1*, *6*, and 7; Wa, lane *2*; Le, lane *3*; and Tu, lanes *8* and *9*), antilymphocyte antibody-negative SLE serum (lane *4*), or normal human serum (lane *5*).

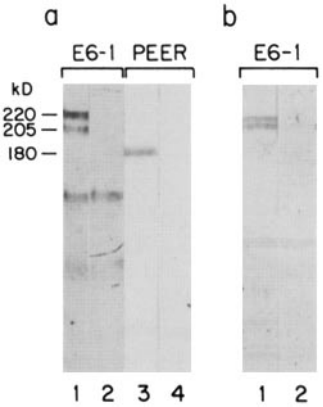


**Figure 2.** Extra-cytoplasmic domain specificity of IgM autoantibodies in SLE sera for different isoforms of CD45. (*a*) WGA eluates of E6-1 or PEER were pre-cleared of CD45 with mAb T191 (even lanes) or control IgG2a (odd lanes) and examined for residual reactivity with SLE IgM by immunoblotting (patient McD, lanes *1* and *2*; patient Tu, lanes *3* and *4*). (*b*) Serum from SLE patient Wa was exhaustively absorbed with packed human erythrocytes, as a control (lane *1*), or viable E6-1 cells (lane *2*) and then examined for residual reactivity with CD45, as described in Materials and Methods.

this molecule, which is identical among all isoforms. Consistent with this interpretation was the observation that exhaustive absorption of SLE sera with viable E6-1 cells removed, in parallel, both IgM antibody that stained CD45 on blots and IgM staining of T cells by indirect immunofluorescence (Fig. 2 *b*).

It is likely that anti-CD45 autoantibodies recognize amino acid sequence determinants, at least in part, because glycosidase F treatment of E6-1 glycoproteins, while reducing the estimated molecular mass of the CD45 isoforms, did not appreciably alter their capacity to be stained with SLE IgM (data not shown). Epitope mapping will be required to define exactly the fine specificity of anti-CD45 autoantibodies, but it can be deduced from the present data that at least some of the reactive epitopes are distinct from those recognized by anti-2H4 on p220/p205 and by UCHL-1 on p180. Thus, of eight SLE sera studied in detail, four reacted only with p220 and p205 CD45RA isoforms (13), but the other four sera reacted with p205 alone (one serum), p220 alone (two sera), p205, p120, and p180 (one serum), and p220, p205, and p190 (one serum). Of interest, the dominant specificity of SLE autoantibodies for CD45RA isoforms appears to be similar to that of mouse mAbs, which also recognize CD45RA epitopes primarily (13).

The anti-CD45 autoantibodies described in the present investigation may contribute to some of the immunologic abnormalities in SLE (5) and other disorders (18), e.g., depletion of functionally important T cell subpopulations (4, 18, 19) and defective T cell activation in response to antigenic stimuli (20, 21). Thus, CD45 is absolutely required for T cells to enter the cell cycle in response to stimulation with antigen (22). Its cytoplasmic domain has protein tyrosine phosphatase activity, which plays an essential role in signal transduction (8, 23). mAbs to different isoforms of CD45 inhibit or enhance lymphocyte activation in vitro (12, 23–25), probably by interfering with the capacity of CD45 to interact with other cell surface proteins in regulating the phosphorylation of molecules important in cellular activation after triggering of the TCR/CD3 complex (9, 10). Experiments to delineate the effects of anti-CD45 autoantibodies on lymphocyte function and to define their relevance to the pathogenesis of SLE will be particularly challenging.

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