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ANTI-LYMPHOCYTE AUTOANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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INTRODUCTION

When I arrived in the Rockefeller University in 1973, the late Professor Henry Kunkel suggested that I begin work on anti-lymphocyte autoantibodies. This has remained an interest of mine ever since, in part because if Henry Kunkel thought that this was an important area, it must be an important area. In my remarks today, I will give a brief overview of the highlights of this system and conclude by discussing some newer data concerning autoantibodies to CD45, the major protein tyrosine phosphatase on T cells and other nucleated hemopoietic cells. These latter observations show promise for finally elevating the potential contribution of anti-lymphocyte antibodies to cellular immune dysfunction in SLE from the realm of phenomenology.

Most patients with SLE develop anti-lymphocyte autoantibodies at some point in their illness. Best studied are cold-reactive IgM lymphocytotoxic autoantibodies, which vary in titer with disease activity status in the same fashion as anti-dsDNA autoantibodies (1, 2). This association with disease flares and lymphopenia has always intrigued me because it forms one line of evidence for a potential role of IgM anti-lymphocyte autoantibodies in T cell depletion and cellular immune dysfunction in SLE. As a group, IgM anti-lymphocyte autoantibodies probably represent a type of natural autoantibody that exhibits a disease-related increase in titer. Their multivalency, together with the local density of reactive antigens on the cell surface, confers a capacity for a variety of immunoregulatory and non-specific physiologic roles in the immune system and in autoimmune disease (3). Although still poorly understood, over the years we have obtained

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evidence for several potential mechanisms by which this could occur: elimination of lymphocytes by complement-mediated lysis (2) and/or opsonization; modulation of surface determinants (4); interaction with soluble products of activated cells (5, 6); and up-regulation or down-regulation by cross-linking cell surface receptors (7). Thus, autoantibodies to lymphocyte surface determinants may constitute one of the pathogenetically significant extrinsic elements that influence the immune system in this disorder.

OBSERVATIONS

Antigenic Specificity

Originally described as "cold-lymphocytotoxins" (8), IgM anti-lymphocyte autoantibodies react broadly with autologous lymphocytes and with lymphocytes from unrelated donors. Their cold-reactivity has been exploited for isolation and purification by elution from cells at warm temperatures and is useful in the operational discrimination of IgM anti-lymphocyte autoantibodies from IgG alloantibodies and relatively more warm-reactive IgG anti-lymphocyte autoantibodies. Early studies in the pre-monoclonal antibody era established a relative specificity of lymphocytotoxic autoantibodies in SLE for T cells, especially thymocytes, and detected interesting specificities for functionally significant T cell subsets (reviewed in reference 9). IgM autoantibodies to B cells from a variety of sources also were described, which are at least partially distinct from those to T cells, as demonstrated in differential absorption experiments using B and T cell lines (1).

The application of monoclonal antibody and T cell cloning technology to characterization of T cell subsets and their function during the 1980s enabled substantial clarification of the nature of autoantibodies to T cells and their potential significance. For example, reactivity of T cells to classic cold-reactive anti-lymphocyte autoantibodies was observed to be dependent primarily on the presence of antigen(s) that are shared by CD4+ and CD8+ subsets, with a superimposed specificity for an additional antigen(s) expressed on CD4+ T cells (10). Activated T cells, which exhibit a higher density of many surface membrane antigens than do resting cells and also express neoantigens, were found to be special targets of autoantibodies (7).

The full spectrum of cell-surface target antigens in SLE, the relative frequency of autoantibodies of a given specificity, and the precise relationship of different autoantibodies to disease expression and cellular immune function are still unknown. Technical difficulties that

have impeded progress here are the very low titer of such antibodies in SLE sera and the confounding element of in vivo absorption, which may remove those antibodies of potentially greatest interest. Nevertheless, considerable information has slowly accrued concerning target antigens in this system: IL-2 receptor (11); β_2 microglobulin (12, 13); HLA Class I heavy chains (14); a DR framework epitope (15); IgD, which functions as an antigen receptor on B cells (16); determinants on plasma membrane molecules that cross-react with histones and DNA (17) or ribosomal P protein (18); B and T cell membrane glycosphingolipids (19); CD45 (20–22); and synthetic T cell receptor (TCR) peptides (23). Cold agglutinins with I,i specificity represent a type of IgM anti-lymphocyte autoantibody because I,i antigens are expressed on lymphocytes, but contribute relatively little to the lymphocytotoxic activity of SLE sera as detected in conventional assays performed at cold temperatures. Although vigorously pursued, neither viral antigens nor anti-viral antibodies have been convincingly implicated as part of the anti-lymphocyte autoantibody system.

Autoantibodies to CD45—Specificity Studies

CD45, an unusually abundant transmembrane protein on hemopoietic cells, functions as a positive growth regulator that is required for antigen-stimulated proliferation of T and B cells (for reviews, see references 24–26). It is encoded by a single gene that generates eight isoforms by alternative splicing of exons 4, 5, 6, and 7, which encode the extracellular domain. The molecular weights of CD45 vary from ~180 kDa for CD45θ (p180; exons 4, 5, 6, and 7 not expressed) to ~220 kDa for CD45α (p220; exons 4, 5, 6, and 7 all expressed). Variability of the extracellular domain also derives from qualitative and quantitative differences in glycosylation patterns of multiple N-linked sites and a highly O-glycosylated region (see reference 22 for review). The intracytoplasmic segment has two internally homologous tyrosine phosphatase domains. This arrangement of the molecule suggests that the cytoplasmic enzymatic activity is under the control of extracellular ligand-binding structures, e.g., CD45θ with CD4 (27). CD45 is a positive regulator of proximal signal transduction following ligation of the TCR and other surface receptors via the tyrosine-phosphorylation circuit. In addition to a crucial role for CD45 in the initiation of signaling, CD45 may be involved in termination of T-cell responses as well (28). Depending on the nature of the activation signals, the experimental conditions of the assays, and the state of lymphocyte activation, different anti-CD45 mAbs profoundly influence T cell function, such as

inhibition or augmentation of T-cell activation. In some cases, such actions of anti-CD45 mAbs have been related to their specificity for oligosaccharides.

That CD45 might be a target of IgM anti-lymphocyte antibodies was suggested initially by studies using the monoclonal antibody anti-2H4 (anti-CD45RA), which showed that patients with active SLE had significantly decreased percentages of circulating T cells bearing the CD4+,2H4+ phenotype (29) and that anti-lymphocyte antibodies from these patients reacted preferentially with the CD4+,2H4+ subset (30). Initial experiments in this area by Seiji Minota, a fellow in our laboratory from Tokyo, identified several high molecular weight isoforms of CD45 on T-cell lines as targets of IgM anti-lymphocyte antibodies (20). Next, the reactive determinants in this system were shown to be extracellular O-linked glycans of CD45, rather than N-linked glycans, linear polypeptide sequences, or the intracytoplasmic tail of this molecule (21). As we examined this system further, we established that IgM anti-CD45 autoantibodies could, indeed, recognize native CD45 expressed on the surface membrane of viable cells, with striking specificity for CD45 from certain mature T-cell lines and activated peripheral blood T-cells, but not primitive T-cell lines, B-cells, or resting peripheral T-cells (22). In addition, evidence was obtained for a relatively frequent reactivity with CD45θ on activated peripheral T-cells.

In further studies of the CD45 glycan specificity of anti-CD45 autoantibodies by Phil Fernsten in our laboratory (31), affinity-purified CD45 from Jurkat cells was digested with a large panel of exoglycosidases, selected to cover the most common terminal residues and linkages. Of these, only digestion of CD45 with α-N-acetylgalactosaminidase eliminated the reactivity of anti-CD45 autoantibodies from most of the sera tested. Relatively few O-glycans typically terminate in α-linked GalNAc residues, the most common being blood group substances A and Sd^a/Cad and the tumor-associated Tn and sialosyl-Tn antigens (32). Blood group substance A and Sd^a/Cad were eliminated as candidate epitopes. In a series of as yet unpublished experiments using purified CD45 from various lymphocyte types and monoclonal antibodies specific for Tn and sialosyl-Tn as probes, reactivity of anti-sialosyl-Tn was essentially restricted to Jurkat CD45 isoforms and the CD45θ (p180) isoform of activated peripheral blood T cells, exactly paralleling reactivity with SLE IgM autoantibodies (Figure 1). Specificity of the SLE anti-CD45 autoantibodies for sialosyl-Tn was confirmed by ELISA (data not shown) and as shown in Table 1, by immunoblot/elution experiments with solid-phase Jurkat CD45 and ovine submaxillary mucin (>90% of glycans express sialosyl-Tn).

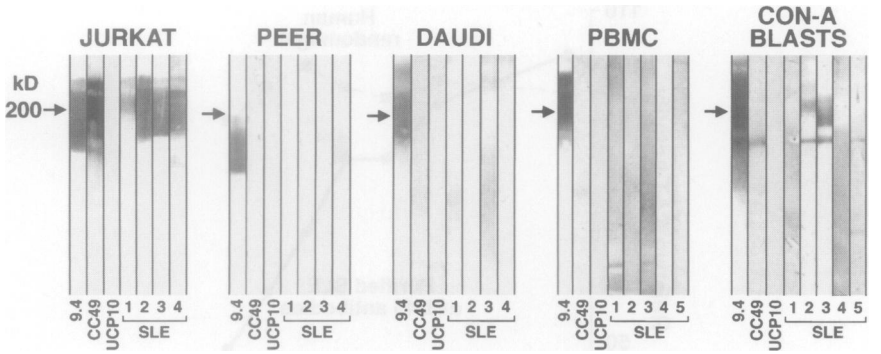

FIG. 1. Immunoblots of purified CD45 from different types of lymphocytes probed with monoclonal antibodies to CD45 (9.4) and sialosyl-Tn (CC49), a control monoclonal immunoglobulin (UCP10), and IgM from SLE patients 1–5. Jurkat is a mature T cell line that expresses higher molecular weight CD45 isoforms. PEER is a T cell line that expresses lower molecular weight CD45 isoforms, including CD45θ (p180), but not the sialosyl-Tn epitope. DAUDI is a B cell line. PBMC and Con-A blasts are normal peripheral blood mononuclear cells in resting state or following 3 days stimulation with Concanavalin A, respectively. Only the CD45θ isoform on Con-A blasts exhibits the sialosyl-Tn epitope.

TABLE 1

Affinity purified autoantibodies to CD45 cross-react with ovine submaxillary mucin (OSM, >90% sialosyl-Tn). ELISA absorbance was determined at 490 nm. Assays used SLE IgM, SLE IgM eluted from solid-phase CD45 or OSM, or normal IgM in combination with 500 ng of CD45 or OSM.

	JURKAT CD45	OSM
Normal	0.02	0.00
SLE 1	1.32	0.62
SLE 1, CD45 eluate	1.29	0.68
SLE 1, OSM eluate	0.48	0.61
SLE 2	0.57	0.28
SLE 2, CD45 eluate	0.52	0.21
SLE 2, OSM eluate	0.42	0.23

Autoantibodies to CD45—Functional Studies

Years ago we demonstrated that SLE anti-lymphocyte autoantibodies directed against then unknown surface antigen(s) on T cell blasts inhibited early phase activation events in the T-cell proliferative response to tetanus toxoid (7). It is now understood that engagement of the T cell receptor (TCRζ/CD3 complex) activates src-family protein-tyrosine kinases (PTKs), $p59^{fyn(T)}$ and, most notably, $p56^{lck}$ and that CD45 plays a critical positive regulatory function in proximal signaling through dephosphorylation of a negative-regulatory tyrosine at their

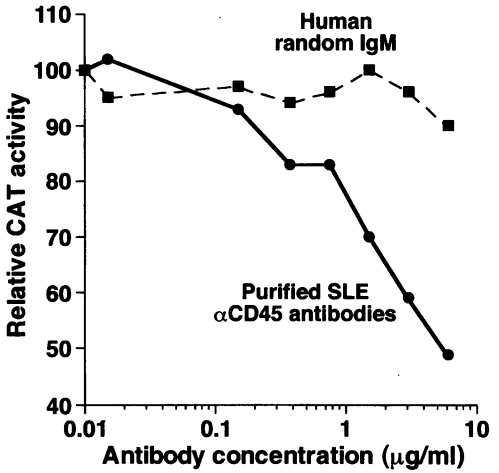

FIG. 2. Purified IgM anti-CD45 autoantibodies from a patient with SLE inhibit in dose-dependent fashion $p21^{ras}$-induced transcriptional activation (measured as % conversion of ^{14}C-chloramphenicol to its acetylated form) in Jurkat cells transfected with $p21^{ras}$-responsive pB4X-CAT reporter plasmid.

C-termini, which up-regulates their PTK activity. Downstream, $p21^{ras}$ activation together with multiple other proximal signaling pathways ultimately couples the TCR to the mitogen-activated protein (MAP) kinase cascade, which regulates the expression of genes encoding IL-2, etc (25, 33).

In very recent experiments by a fellow in my laboratory, Jan Czyzyk, SLE IgM autoantibodies to CD45 have been shown to inhibit both IL-2 production and $p21^{ras}$ activation in T-cells (unpublished data). Following stimulation of Jurkat cells with PMA + OKT3, addition of SLE IgM anti-CD45 autoantibodies (1 μg/ml) resulted in partial reduction (24–50%) in IL-2 secretion, as quantitated in a standard sandwich ELISA. Next, using Jurkat cells transfected with $p21^{ras}$-responsive pB4X-CAT reporter plasmid and stimulated with PMA + OKT3, affinity-purified SLE IgM anti-CD45 autoantibodies inhibited $p21^{ras}$-induced transcriptional activation (measured as % conversion of ^{14}C-chloramphenicol to its acetylated form) in a dose-dependent fashion (Figure 2). These latter findings are reminiscent of those recently reported in B-cells, where ligation of CD45 by anti-CD45 mAb to the p220 CD45 isoform down-regulates $p21^{ras}$ activation following engagement of the B-cell antigen receptor (34), and presumably involves effects on $p56^{lck}$ activation (35). Our working hypothesis is that pentameric IgM anti-CD45 autoantibodies cross-link CD45, thereby preventing CD45 from being where

it should normally be for optimal signal transduction and IL-2 secretion.

CONCLUSIONS

IgM anti-lymphocyte autoantibodies, although of relatively low titer, are almost universally present in patients with active SLE and exhibit interesting specificities for CD45 and other surface structures of functional importance in the immune response. A considerable body of data suggests that anti-lymphocyte autoantibodies contribute to lymphopenia and cellular immune dysfunction in this disorder. Their exact contribution in this regard remains obscure, however. Nevertheless, our recent data concerning the specificity of autoantibodies for a sialosyl-Tn epitope on CD45θ expressed by activated peripheral T-cells and the inhibitory effect of such autoantibodies on proximal T cell signaling and IL-2 secretion are beginning to clarify this issue.

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DISCUSSION

Sergent, Nashville: Patients with lupus who develop AIDS usually show a progressive improvement in their lupus and sometimes complete disappearance of the disease as the CD4 level falls. Based on that, you would think that anti-lymphocyte antibodies that are specific for CD4 cells would improve clinical and immunologic findings in lupus, yet you have shown in many studies that there is a direct correlation between antibody level and lupus activity. Is this just a quantitative phenomenon? Is it that the anti-CD4-coated cells don't interfere with T-cell function to the same degree that AIDS does with CD4 function? Is there some qualitative phenomenon going on?

Winfield: Your question, John, points out the importance of CD4 cells in providing help for the immune response, which is totally out of control in active lupus. It also raises the issue of whether anti-CD45 autoantibodies, which clearly can influence CD4 function, contribute in a negative way to the pathogenesis of lupus, or actually are helpful, i.e. they may represent an abortive attempt at homeostasis. Despite working on this for a long time, I still don't know whether these are bad antibodies or good antibodies.