# Antilymphocyte autoantibodies generate T cell–C4d signatures in systemic lupus erythematosus

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**T cells bearing C4d, a complement activation product (CAP), have been shown to be highly sensitive and specific as diagnostic biomarkers for systemic lupus erythematosus (SLE). T cells bearing C4d are also functionally abnormal, suggesting a role for cell-bound CAPs in lupus pathogenesis. However, the mechanism responsible for generation of T-C4d has not been determined. The purpose of this cross-sectional and prospective study was to investigate the potential role of anti–T-cell autoantibodies in the generation of the T cell-bound C4d (T-C4d) signatures in SLE. Briefly, T cells from patients with SLE (n = 326), patients with other inflammatory diseases (n = 185), and healthy controls (n = 48) were characterized for surface deposition of either or both of C4d and immunoglobulin (Ig) by flow cytometry. In vitro phenotype transfer experiments were performed to characterize Ig from patients with SLE for the capacity to generate T-C4d signatures in vitro. The results demonstrate that individual patients with SLE harbor specific signatures reflecting the presence of either or both of C4d and Ig on their T cells and T-cell subsets. In addition, SLE patient-specific signatures can be transferred in vitro to normal T cells by exposure to Ig purified from the signature donor. Complement activation does not proceed through the generation of C5b-9 (membrane attack complex) or cellular lysis, and T-C4d does not correlate with lymphopenia. In conclusion, these results suggest that patient-specific T-C4d signatures are generated by anti–T-cell autoantibodies that trigger sublytic complement activation, a previously unrecognized pathway in lupus pathogenesis. (Translational Research 2014;164:496–507)**

**Abbreviations:** ADCC = antibody-dependent cellular cytotoxicity; ALA = antilymphocyte autoantibodies; CB-CAPs = cell-bound complement activation products; C4d = complement C4 activation product C4d; Ig = immunoglobulin; T-C4d = T cell–bound C4d; SLE = systemic lupus erythematosus

## INTRODUCTION

Systemic lupus erythematosus (SLE), the prototypic systemic autoimmune disease, is characterized by myriad immune abnormalities including excessive autoantibody production, activation of the complement system, lymphocyte dysfunction, and lymphopenia.[1-4] On activation of the complement system, proteolytic cleavage of C3 and C4 results ultimately in the generation of C3d and C4d fragments that contain the highly reactive thioester moiety and can bind covalently to the surfaces of pathogens, cells, or immune complexes.[5,6] We have recently reported that significant levels of C4d are present specifically on the surfaces of erythrocytes,[7] reticulocytes,[8] platelets,[9] and lymphocytes[10] of patients with SLE. A recent multicenter study validated cell-bound complement activation products (CB-CAPs) as diagnostic biomarkers for

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## AT A GLANCE COMMENTARY

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#### Background

Anti-T cell autoantibodies were first reported in the 1970's, however their role in the pathogenesis of SLE has not been determined. T cells bearing complement fragment C4d (T-C4d) have been shown to be highly sensitive and specific for a diagnosis of lupus, suggesting a link between anti-T cell autoantibodies and T-C4d.

#### Translational Significance

This study identifies a previously unrecognized molecular and cellular pathway by which anti-T cell autoantibodies trigger sublytic complement activation on T cells in patients with SLE. Deposition of T-C4d through this pathway may render the target cells dysfunctional yet viable and circulating, suggesting a fertile source to mine for lupus biomarkers and targets for therapeutic intervention.

lupus,[11] and additional reports have demonstrated their significant potential as biomarkers of lupus disease activity and stratification.[12,13]

In addition to their roles as lupus biomarkers, CB-CAPs have been shown to confer functional abnormalities to circulating cells such as erythrocytes and T lymphocytes, suggesting a role in lupus pathogenesis.[14-16] Elucidation of the cellular and molecular events whereby CB-CAPs are generated may lead to the identification of potential therapeutic targets by preventing, disrupting, or neutralizing the downstream effects of CB-CAP generation. One of the most intriguing potential links between CB-CAPs and lupus pathogenesis is the longstanding yet poorly understood observation that patients with SLE harbor circulating antilymphocyte autoantibodies (ALAs).

ALAs in patients with SLE, particularly those specific for T cells, were discovered in the 1970s.[17-22] Since then, numerous efforts have been made to characterize these ALAs. However, their role in disease pathogenesis has remained uncertain.[23] Two primary types of anti–T-cell autoantibodies in SLE have been described previously. First, cold-reactive immunoglobulin M (IgM) antibodies, which bind optimally to T cells at 4°C, have been reported as common in patients with SLE.[24,25] However, the in vivo significance of these antibodies is unclear because of the thermal difference between in vitro assays and in vivo pathogenic molecular and cellular mechanisms. Second, warm-reactive immunoglobulin G (IgG) anti–T-cell autoantibodies in lupus have been reported.[25,26] These antibodies have been shown to have heterogeneous specificities against a variety of T-cell surface molecules including CD3, CD4, CD45, and interleukin (IL)-2R.[24,27-30]

Two distinct roles for these IgM vs IgG anti–T-cell autoantibodies in lupus pathogenesis have been suggested, both of which involve destruction of the cellular targets. IgM is 500-fold more effective in activation of the classical complement pathway, suggesting possible lytic attack of T cells.[31,32] However, if binding of these cold-reactive IgM molecules only occurs at low temperatures, this would eliminate this possibility in vivo. In addition, the presence of IgM anti–T-cell autoantibodies has not been shown to correlate with lymphopenia in SLE.[33] IgG are less potent complement activators; however, their warm reactivity makes such an in vivo mechanism at least feasible.[34,35] Other potential roles for anti–T-cell IgG have been suggested including antibody-dependent cellular cytotoxicity (ADCC)[36,37] and modulation of T cell signaling and gene expression.[29]

Some reports have suggested that T lymphocyte dysfunction in lupus might be triggered by circulating IgM and IgG anti–T-cell autoantibodies rather than because of intrinsic defects, although the 2 possibilities are not mutually exclusive.[23] Collectively, these prior reports have suggested that anti–T-cell autoantibodies are present in some patients with SLE and elucidation of their potential role(s) in disease pathogenesis should consider isotype, thermal amplitude of binding and cytotoxicity, and antigenic specificity.

We hypothesized that anti–T-cell autoantibodies in SLE may be responsible for generating T cell–bound C4d (T-C4d) patient-specific signatures through sublytic complement activation, and this may be an important but previously unrecognized link in the complex interplay between autoantibodies and complement activation in lupus pathogenesis.

## METHODS

**Study participants.** All study participants were aged 18 years or older and provided written informed consent. No one was excluded based on gender or ethnicity. Ethnicity was self-reported by study participants. The Institutional Review Boards of the University of Pittsburgh and the West Penn Allegheny Health System approved this study.

***Patients with SLE.*** Three hundred twenty-six patients who met the American College of Rheumatology classification criteria for definite SLE[38] were recruited and followed during routine visits to the University of Pittsburgh Lupus Patient Care and Translational