

BASIC SCIENCE FOR THE CLINICIAN

CD154 and Lupus

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Running title: CD40 Ligand and lupus

Key words: systemic lupus erythematosus, CD40-ligand, CD154, monoclonal
antibody, cyclosporin A

Abstract

CD154 (CD40 ligand) is expressed as a soluble cytokine and as a homotrimeric type II transmembrane protein on the surface of activated CD4 T lymphocytes. The interaction of CD154 with its receptor, CD40, on B cells is critical for B cell growth, differentiation, and antibody isotype switching. Because of its multiple effects in the immune system, expression of CD154 is normally very tightly regulated. However, in autoimmune diseases, such as systemic lupus erythematosus (SLE), CD154 is over-expressed and contributes to disease pathology. Although monoclonal antibody therapies directed against CD154 have been successful at treating disease in murine models of SLE, human trials have been disappointing to date. Therefore, approaches based on correcting the dysregulation of CD154 in SLE are being pursued. Studies of the normal regulation of CD154, and of the dysregulation of CD154 in SLE, are still in their infancy but clues to the pathways and proteins involved are being reported. Ultimately, the ability to manipulate CD154 expression may prove useful for correcting the defect(s) in autoimmune disorders, such as SLE, and it may also aid in treating a whole host of other diseases where CD154 is thought to play a role.

CD154 in the Immune Response

CD154 (formerly known as CD40 ligand) is a member of the tumor necrosis factor superfamily, and it is rapidly expressed as a cell surface activation antigen and as functionally active soluble trimers in the serum (1). CD154 is primarily expressed by activated CD4 T lymphocytes for brief periods of time, up to 24 hours after stimulation (2). CD40, which is constitutively expressed by B lymphocytes, macrophages, dendritic cells, and many other cell types, is the only known partner receptor for CD154 (3). By interacting with CD40, CD154 triggers a pleiotropic immune response.

B cells depend on CD154 stimulation for growth, development, and antibody isotype class switching (4). However, recent studies *in vitro* suggest that CD154 may not be absolutely required for the generation of certain immunoglobulin isotypes (5). In addition, the CD154-CD40 interaction is critical for germinal center formation (6). CD154 triggering of dendritic cells also stimulates interleukin-12 production and indirectly primes CD8 T cell effector function (6). CD154 is important for optimal T cell dependent antibody responses as well (7). It is no wonder that CD154 has been considered by some as the, "Center of the Immune Universe" (8).

Transcriptional Regulation of CD154

Because CD154 drives multiple effector functions throughout the immune system, CD154 expression on CD4 T cells is normally very tightly regulated. Like many cytokines, expression of CD154 is primarily regulated at the level of transcription. The human CD154 transcriptional promoter has been partially characterized and was found to be positively regulated by the cyclosporin A (CsA)-sensitive transcription factor family, nuclear factor of activated T cells (NFAT) (9). Some NFAT proteins are pre-existing in the cytoplasm of T cells and rapidly transit to the nucleus upon sustained intracellular calcium levels following T cell activation (10). Recently, several other transcription factors have also been implicated in the regulation of the CD154 promoter (11).

In addition to the CD154 transcriptional promoter, two others have been identified by DNase I hypersensitivity site mapping (11). A novel GATA- and NFAT-regulated transcriptional enhancer element has been identified just upstream (5') of the CD154 transcriptional promoter (12), and a newly described NFκB-responsive enhancer has recently been reported within and immediately downstream (3') of the CD154 3' untranslated region (13). The 3' untranslated region also serves to regulate CD154 expression at the level of mRNA stability, and a novel RNA binding protein may specifically regulate CD154 expression (14). Thus, although the regulation of CD154 in primary human CD4 T cells under normal circumstances is complex, progress has been made in defining this regulation (11).

CD154 and Disease

Abnormal or dysregulated expression of CD154 by CD4 T cells, and by and other cell types that do not normally express CD154, has been implicated in the pathogenesis of a wide array of diseases ranging from atherosclerosis to Alzheimer disease (11). Dysregulated expression of CD154 has also been associated with a variety of autoimmune disorders from rheumatoid arthritis to inflammatory bowel disease (Table I). However, the autoimmune disease best characterized in terms of CD154 dysregulation is systemic lupus erythematosus (SLE).

CD154 Expression in SLE

Murine models of SLE first reported that CD154 was over-expressed on T cells from lupus-prone strains of mice (15). Furthermore, blockade of the CD154-CD40 interaction in mice with lupus has been shown to delay and decrease the incidence of glomerulonephritis (15, 16). Moreover, treatment with anti-CD154 monoclonal antibody prolonged the survival of mice with established lupus nephritis (17). Taking another approach, two independent groups of researchers recently showed that over-expression of CD154 in mice *in vivo* triggered autoantibody production (18) and lead to a lupus-like disease (19). Thus, CD154 over-expression appears to be a major culprit in the pathogenesis of disease in murine models of SLE.

Over-expression of CD154 on CD4 T cells has also been demonstrated in human SLE. Two independent labs reported that *in vitro* activated peripheral blood T cells from patients with SLE expressed increased and prolonged (over 24 hours) levels of CD154 (Table II) (20, 21). As controls, two different activation markers, CD25 and CD69, were expressed at similar levels after mitogen-induced activation of T cells from controls and patients with SLE (20, 21). Furthermore, CD154 has been found to be over-expressed immediately *ex vivo* on T cells from patients with SLE (20, 22). Similar results for baseline and *in vitro*-activated hyper-expression of CD154 on T cells have also been found in children with SLE (23). Importantly, the increased expression of CD154 on SLE T cells was demonstrated to induce higher levels of CD80 on co-cultured B lymphocytes (20) and to produce pathogenic-variety antinuclear antibodies *in vitro* (21). It is currently unclear whether the increased/prolonged expression of CD154 helps to break tolerance of autoreactive B cells by increasing their survival and/or by providing growth and differentiation signals. Nevertheless, it does seem likely that over-expression of CD154 on T cells of patients with SLE contributes to disease pathogenesis.

In addition to the increased expression of CD154 on CD4 T cells, patients with SLE were also found to have ectopic expression of CD154 on B cells, CD8 T cells, and CD4⁻, CD8⁻ T cells (Table II) (21, 22). Furthermore, soluble CD154 (sCD154) has been reported to be elevated in the serum of patients with SLE compared to controls (Table II) (24-26). The levels of sCD154 correlated with dsDNA titers and disease activity scores in patients with SLE (26), and the elevated sCD154 levels were found to be functional in that they were shown to increase expression of accessory molecules on B cells (25, 26). Interestingly, a correlation between sCD154 levels and coronary artery

calcification was recently noted in adults with SLE (24). Elevated levels of sCD154 and surface bound CD154 have also been shown to be a marker for unstable angina (27). Therefore, elevated sCD154 levels may serve as an indicator to screen for coronary artery disease in patients with SLE.

Regulation of CD154 in SLE

In one study, levels of sCD154 but not surface CD154 on T cells were found to correlate with CD154 mRNA levels from SLE T cells (26). This does not address, however, whether or not the increased CD154 is due to differences in CD154 transcription and/or CD154 mRNA stability. Preliminary data argues for both increased CD154 mRNA stability (28) and increased CD154 transcription (23) in T cells from individuals with SLE. In addition, CD4 T cell lines generated from individuals with SLE were found to have increased activity of the mitogen-activated kinase, ERK, leading to prolonged CD154 expression (29). ERK pathways certainly can contribute to increased transcription factor activity but they may also alter protein levels in a non-transcriptional manner. Interestingly, CsA, the powerful NFAT inhibitor, markedly decreased CD154 expression up to 18 hours, but only modestly inhibited expression at later time points (29). Another indirect argument for the role of transcription in the abnormal expression of CD154 in lupus, comes from a study that showed that a global transcriptional regulator, trichostatin A, was able to reverse the abnormal expression of CD154 in SLE T cells (30). It may well be that differences in both transcription and mRNA stability may lead to dysregulated CD154 expression at different time points post T cell activation in patients with SLE.

Strategies to Target CD154 in SLE

Until, the precise pathway(s) is identified that leads to dysregulated CD154 expression in SLE, it may be difficult to treat this aspect of lupus. An obvious candidate for treatment is CsA, which clearly inhibits CD154 expression *in vitro*. However, because the levels of CsA needed to achieve inhibition *in vivo* (31) may lead to substantial nephrotoxicity, this is not optimal for treating those with SLE and glomerulonephritis. In deed, CsA is rarely used to treat pediatric SLE (32). Also, since CsA can inhibit other transcription factors in addition to NFAT proteins in primary human CD4 T cells (33, 34), specific NFAT inhibitors may be more promising (31, 35). Similar to studies in mice, monoclonal antibodies directed against human CD154 have been tested in treating SLE.

Unfortunately, one studied led to unanticipated thromboembolic complications and was discontinued (36). Recently, another independent phase II, double-blinded trial using a different humanized anti-CD154 monoclonal antibody to treat patients with active SLE was well tolerated but did not show any efficacy over placebo (37). Hypothetically, the dose of an antibody required to block the CD154-CD40 interaction *in vivo*, and the subsequent SLE disease pathology, may leave the patient in an immunodeficient state similar to those with Hyper IgM syndrome, who are born with a defect in CD154 expression (4). Ultimately, a treatment(s) targeted at the upstream

pathway(s) leading to CD154 dysregulation in SLE may be necessary to avoid unnecessary immunodeficiency as a side effect of therapy.

Acknowledgments

This work was supported in part by the Kahn Foundation for lupus research, a fellowship from the Howard Hughes Medical Institute, and by grants from the National Institutes of Health, the Arthritis Foundation, the Dorough Lupus Foundation, the Arthritis National Research Foundation, the Elizabeth Glaser Pediatric AIDS Foundation, and the Mary L. Smith Charitable Trust. The author would like to thank Dr. Terri H. Finkel (The Children's Hospital of Philadelphia, PA) for critical review of the manuscript.

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TABLES

Table I. Autoimmune Diseases Associated with CD154

<u>Disease</u>	<u>Reference</u>
Inflammatory bowel disease	(38)
Rheumatoid arthritis	(39)
Systemic lupus erythematosus	(40)
Systemic sclerosis	(41)

Table II. Varieties of abnormal CD154 expression in SLE

<u>Abnormality</u>	<u>References</u>
1. Prolonged expression on CD4 T cells after activations	(20,23)
2. Higher levels of CD154 expression cells	(20,21,23)
3. Increased percentage of CD4	(21)
4. CD154 expression of unmanipulated CD4 T cells <i>ex vivo</i>	(20,21,23)
5. Increased levels of sCD154 in the serum	(24-26)
6. <i>De Novo</i> CD154 expression on unusual cell types (eg., CD8 T cells)	(21,22)