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Murine AIDS requires CD154/CD40L expression by the CD4 T cells that mediate retrovirus-induced disease: Is CD4 T cell receptor ligation needed?

Wen Li [a], William R. Green [a,b,*]

[a] *Department of Microbiology and Immunology, Dartmouth Medical School, USA*
[b] *Norris Cotton Cancer Center, Dartmouth Hitchcock Medical Center, Lebanon, NH 03756, USA*

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Abstract

LP-BM5, a retroviral isolate, induces a disease featuring an acquired immunodeficiency syndrome termed murine AIDS (MAIDS). Many of the features of the LP-BM5-initiated disease are shared with HIV/AIDS. Our lab has shown that the interaction of B and CD4 T cells that is central to MAIDS pathogenesis requires ligation of CD40 on B cells by CD154 on CD4 T cells. Despite this strict requirement for CD154 expression, whether CD4 T cell receptor (TCR) occupancy is essential for the induction of MAIDS is unknown. To block TCR engagement, Tg mouse strains with monoclonal TCR of irrelevant peptide/MHC specificities, all on MAIDS-susceptible genetic backgrounds, were tested: the study of a panel of TCR Tg CD4 T cells controlled for the possibility of serendipitous crossreactive recognition of virus-associated or induced-self peptide, or superantigen, MHC complexes by a given TCR. The results argue that TCR engagement is not necessary for the induction of MAIDS.

Keywords: Murine AIDS (MAIDS); LP-BM5 retrovirus; Pathogenesis; Immunodeficiency; CD4 T cells; CD154; CD40; T cell receptor (TCR); Signal 1; Transgenic (Tg)

Introduction

CD40 ligand (CD40L or CD154) is a 33-kDa transmembrane glycoprotein that is transiently expressed on the surface of activated T cells, predominantly CD4 T cells. Its receptor, CD40, is found on B cells, dendritic cells, monocytes, and some other cell types. The CD154/CD40 system is crucially involved in immunity because cognate interactions between CD154 and CD40 generate intra- (through CD40) and inter-cellular signals that result in up-regulation of a variety of cell surface and soluble molecules that ultimately impact humoral and cellular immunity, as well as inflammation and other disease processes (e.g. Laman et al., 1996; Danese et al., 2006). For example, CD154 and CD40 are overexpressed in both forms of inflammatory bowel disease (IBD) (Liu et al., 1999; Danese et al., 2006), Crohn's disease (CD) (Battaglia et al., 1999), and ulcerative colitis (Polese et al., 2002). These studies and others strongly indicate that the CD154/CD40 pathway plays a key pathogenic role in intestinal inflammation. A number of studies have focused on the impact of CD154/CD40 interactions on the immunopathogenesis of other autoimmune diseases, such as systemic lupus erythematosus (SLE) (Desai-Mehta et al., 1996; Koshy et al., 1996), rheumatoid arthritis (RA) (MacDonald et al., 1997), multiple sclerosis (Gerritse et al., 1996), and Graves' disease (Faure et al., 1997); as well as on allograft rejection (Reul et al., 1997).

The interaction of CD154 and CD40 is not restricted to the regulation of immune responses and dysfunction resulting in autoimmunity, but also often plays an important role in the development of effector cells that function at local sites of infection and inflammation (Reichmann et al., 2000). In vivo studies have indicated the important role of CD40 ligation by CD154 in thymus-dependent humoral immune responses and germinal center formation (Allen et al., 1993; Xu et al., 1994; Liu et al., 1999). CD154 also is a critical molecular player in

* Corresponding author. Department of Microbiology and Immunology, Dartmouth Medical School, 603W Borwell Research Building, One Medical Center Drive, Lebanon, NH 03756, USA. Fax: +1 603 650 6223.
E-mail address: william.r.green@dartmouth.edu (W.R. Green).

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antigen-specific T-cell responses. Thus, adoptively transferred antigen-specific CD4 T cells lacking CD154 failed to expand upon antigen challenge of the recipients, showing that expression of CD154 is required for in vivo priming of CD4 T cells and therefore for the initiation of specific T-cell immune responses (Grewal et al., 1995). Similarly, $CD154^{-/-}$ mice display a deficiency in T cell-dependent macrophage-mediated immune responses (Stout et al., 1996). Thus, CD154 ligation of CD40 is important in the protective cell-mediated immune responses conferred by T cell-directed activated macrophage effector function against intracellular parasite infections such as *Pneumocystis, Listeria monocytogenes, Leishmania* (Grewal et al., 1997; Soong et al., 1996; Campbell et al., 1996), and a *T. gondii*-driven experimental model of acute ileitis (Li et al., 2002). CD154/CD40 interactions are also required for the priming and expansion of antigen-specific CD4 T cells and in the induction of co-stimulatory activity on antigen-presenting cells (APCs) (Grewal et al., 1997). CD154/CD40 binding and signaling are also well documented to have a key role in APC maturation and survival in the induction of adaptive immune responses, particularly the activation of naïve T cells by dendritic cells (Grewal et al., 1997; Caux et al., 1994; Peguet-Navarro et al., 1995; Cella et al., 1996).

The LP-BM5 retroviral isolate consists of a pathogenic defective murine retrovirus (BM5def) that requires replication-competent ecotropic helper viruses (e.g. BM5eco) for its entry into cells and spread in vivo. The ensuing murine acquired immunodeficiency syndrome, termed murine AIDS (MAIDS) (Latarjet and Duplan, 1962; Mosier et al., 1985), exhibits many features shared with HIV-induced disease in man (Aziz et al., 1989; Jolicoeur, 1991; Morse et al., 1992; Tayar et al., 1999; Casabianca et al., 2003). Similarities include early onset hypergammaglobulinemia (hyper-Ig), splenomegaly, and lymphadenopathy; dependence on CD4 T cells for initiation of disease; loss of CD4 T cell function; severely depressed T and B cell responses; increased susceptibility to infection and death when exposed to normally nonpathogenic microorganisms; and the development of terminal B-cell lymphomas. Although there are also differences between MAIDS and human AIDS, the study of MAIDS, as the most commonly used murine system of retrovirus-induced immunodeficiency, offers many advantages. Thus, the availability of informative inbred, Tg, knockout, and other mouse strains, and ability to do in vivo pathogenesis experiments allows direct investigation of the cellular and molecular mechanisms required for the development of retroviral pathogenesis, including the associated immunodeficiency.

Both CD4 T cells and B cells are required for disease induction and progression. Severe combined immunodeficient (SCID) mice (lacking mature T and B cells) do not develop MAIDS (Simard et al., 1997). B6 mice that were depleted of B cells from birth by chronic administration of rabbit anti-mouse IgM antibodies (μ-suppressed) are resistant to LP-BM5 MAIDS (Cerny et al., 1990). It has been reported that the main targets of initial LP-BM5 retrovirus infection are B cells (Kim et al., 1994; K. Green, unpublished data), and to some extent macrophages (Cheung et al., 1991) and T cells (Kim et al., 1994; K. Green, unpublished data). However, B-cell infection by itself is not progressive and does not lead to disease in the absence of mature CD4 T cells (Klinman and Morse, 1989; Cerny et al., 1990). Indeed, an early hyperactivity of CD4 T cells may contribute to the pathogenesis of MAIDS (Mosier et al., 1987; Yetter et al., 1988). Thus, in vivo depletion of CD4 T cells by treatment with the anti-CD4-specific mAb GK1.5, before LP-BM5 infection rendered susceptible B6 mice resistant to the development of MAIDS (Yetter et al., 1988). Similarly, congenitally-athymic nude B6 mice become infected but do not develop significant LP-BM5 induced MAIDS (Mosier et al., 1987). However, if reconstituted with CD4 T cells from B6 donor mice, B6.nude recipients develop MAIDS upon LP-BM5 infection (Yetter et al., 1988; Giese et al., 1994). In contrast, by comparing the adoptive transfer of T cell subsets into B6.nude mice, it was demonstrated that CD8 T cells are not required for LP-BM5 induced pathogenesis (Green et al., 2001).

These and other results suggested that an interaction between B and CD4 T cells is critical for the pathogenesis of MAIDS. Thus, in additional adoptive transfer experiments, our lab showed that if B6.nude recipients were reconstituted with $CD154^{+/+}$, but not $CD154^{-/-}$, CD4 T cells, they converted to disease susceptibility (Green et al., 2001). Reciprocally, with regard to the cell type interacting with $CD154^{+}$ CD4 T cells, B6 $CD40^{-/-}$ mice, which are resistant to LP-BM5-induced MAIDS (Green et al., 2001; Yu et al., 1999), became susceptible to LP-BM5-induced disease after reconstitution with highly purified wild-type (w.t.) ($CD40^{+}$) B cells, but not after receiving purified wild-type dendritic cells (DC) or a combined $CD40^{+}$ population composed of DC and macrophages obtained from B6.SCID mouse donors (Green et al., 2001). Indeed, by F2 genetic crossing and screening to generate a B6.$CD40^{-/-}$ SCID recipient, combined transfer of only w.t. ($CD154^{+}$) CD4 T cells and w.t. ($CD40^{+}$) B cells allowed for LP-BM5 infection to cause MAIDS (K. Green, unpublished data). In a follow-up study, we demonstrated that B6 mice deficient for both the CD80/CD86 (B7.1/B7.2) co-stimulatory ligands are susceptible to LP-BM5 induction of MAIDS (Green et al., 2002). These results on the APC (B cell) expressed B7 ligands, coupled with data from another laboratory on the CD80/CD86 receptor-CD4 T cell expressed CD28 (Morawetz et al., 1998), suggested that CD28/CD80-CD86 interactions are not absolutely required for the initiation of MAIDS or the commitment to disease progression. Although the classic upregulation of CD80/CD86 after CD40 ligation is thus not necessary, we have used a panel of chimeric CD40 Tg mice on the $CD40^{-/-}$ background to confirm that CD40 signaling is needed for MAIDS induction and is differentially mediated by TRAF binding to the TRAF 6 site (vs the TRAF 2/3/5 site) on the CD40 cytoplasmic tail (Green et al., 2004). Collectively, these findings provide clear evidence that activated CD4 T cell CD154 ligation of B cell CD40 and downstream signaling events are required for MAIDS (Green et al., 2001, 2004).

In view of the absolute requirement for CD154 expression, it is not clear, however, whether there is a role for CD4 T cell