

## CD40 Ligand/CD40 Deficiency

E.		Castigli
R.		Fuleihan
N.		Ramesh
E.		Tsitsikov
A.		Tsytsykova
R.S.		Geha

Division of Immunology, Children's Hospital, Boston, Mass., USA

### Key Words

CD40  
CD40 ligand  
Isotype switching  
HIGMX-1  
CD40 knockout

### Abstract

CD40 is a surface antigen expressed on B cells. The CD40 ligand (CD40L) is expressed on activated T cells. Interaction between CD40 and CD40L is critical for proliferation and isotype switching in the context of a response to a T-cell-dependent antigen. Patients with X-linked hyper-IgM syndrome (HIGMX-1) in their CD40L gene are unable to switch from IgM to IgG, IgA and IgE. Mice with a disrupted CD40 gene fail to undergo isotype switching to T-cell-dependent antigens but respond normally to T-independent antigens.

Correspondence to: Dr. R. S. Geha, Division of Immunology, Children's Hospital, 300 Longwood Avenue Boston, MA 02115 (USA)

### Introduction

Interactions between the B cell antigen CD40 which is expressed on B cells and its ligand CD40 which is expressed on activated T cells play an important role in T-cell-driven isotype switching [1]. The process of isotype switching has been shown to require two signals, the first being provided by T-cell-derived cytokines which determine which isotype will be targeted for switch recombination [2]. The second signal is provided by CD40 interaction with its ligand which results in deletional switch recombination involving IgM and the gene for the isotype targeted by the cytokine. In the case of IgE, interleukin (IL)-4 targets the IgE gene for switch recombination, and CD40 engagement by its ligand on activated T cells results in switch recombination from IgM to IgE.

The role of the CD40 ligand-CD40 axis in B cells in immunoglobulin class switching is illustrated by the finding that CD40 ligand deficiency forms the basis for X-linked hyper-IgM syndrome (HIGMX-1) [3]. In this review, we will briefly recapitulate our current knowledge of mutations in the CD40 ligand, will present data on the control of CD40 ligand expression, and on the CD40 knockout mice generated in our laboratory, and will discuss the immunological abnormalities in these mice.

### Ligand Expression is Developmentally Regulated in Human Thymocytes

The ligand for CD40 is expressed on activated T lymphocytes and delivers contact-dependent activation signals to B lymphocytes. We have investigated the developmental expression of CD40 ligand by examining CD40 ligand expression in human thymocytes. CD40 ligand was not

detectable on resting immature double positive (CD4+/CD8+) or resting mature single-positive (CD4+/CD8- or CD4-/ CD8+) thymocytes. Immature thymocytes failed to express CD40 ligand on their surface after stimulation with phorbol ester and ionophore, whereas stimulated CD4+/CD8- mature thymocytes expressed CD40 ligand. Northern blot analysis revealed that CD40 ligand mRNA was detectable in activated mature single-positive thymocytes. CD40 ligand expression in mature thymocytes was decreased in intensity as compared to peripheral blood T cells. CD40 ligand expression by mature thymocytes was associated with the capacity to drive immunoglobulin isotype switching in purified B cells. These results suggest that the ability to express CD40 ligand is acquired late in thymocyte development and that mature thymocytes are capable of providing B cells with the activation signals necessary for immunoglobulin isotype switching.

© 1995 S.KargerAG, Basel 1018-2438/95/1073-0037 \$8.00/0

#### The Expression of CD40 Ligand on Newborn Lymphocytes

Immune responses in newborn lymphocytes show a defect in isotype switching from IgM to IgG and IgA. Immunoglobulin isotype switching in B lymphocytes requires a contact-dependent signal from T lymphocytes which is delivered by the ligand for the B cell surface antigen CD40. We investigated the capacity of newborn lymphocytes to express the CD40 ligand and to undergo CD40-ligand-dependent immunoglobulin isotype switching. After stimulation by phorbol ester and ionomycin, newborn lymphocytes expressed markedly decreased amounts of CD40 ligand on their surface compared to normal adult lymphocytes. Northern blot analysis of mRNA derived from activated cord blood lymphocytes also revealed markedly decreased amounts of CD40 ligand mRNA. Decreased expression of CD40 ligand in newborn lymphocytes was associated with a severely decreased ability to undergo T-cell-dependent immunoglobulin isotype switching. Newborn lymphocytes synthesized little or no detectable IgE in response to T-cell-dependent stimulation by IL-4 but synthesized IgE in response to T-cell-independent stimulation by CD40 monoclonal antibody and IL-4 [4]. These results indicate that decreased expression of CD40 ligand in newborn lymphocytes may be the underlying cause of deficient immunoglobulin isotype switching in newborns.

#### Immunological Function in CD40-Deficient Mice Generated by Recombination-Activating Gene 2 (RAG2)-Deficient Blastocyst Complementation

To study the role of the B cell antigen CD40 in immune responses, embryonic stem (ES) cells in which both copies of the CD40 gene had been disrupted by homologous recombination were injected into RAG2-deficient blastocysts to generate chimeras in which all mature lymphocytes are derived from CD40-deficient ES cells. T and B cell number and phenotype were normal in the CD40-/- chimeras. However, B cells failed to proliferate and undergo isotype switching in vitro in response to soluble CD40 ligand. + IL-4 but responded normally to lipopolysaccharide (LPS) + IL-4. CD40-/- chimeras completely failed to mount an antigen-specific antibody response or to develop germinal centers following immunization with the T-dependent (TD) antigen keyhole limpet hemocyanin (KLH). In contrast, CD40-/- mutant mice responded normally to the T-independent (TI) antigens TNP-LPS and TNP-Ficoll. The most noticeable alteration in the serum immunoglobulin levels of young (6- to 8-week old) CD40-/- animals was an absence of IgE and a severe decrease of IgG 1 and IgG2a. Serum IgM and IgG3 levels were normal and serum IgG2b and IgA levels were partially decreased [5]. These results confirm the essential role of CD40/CD40 ligand interactions in the antibody response to TD antigens and in isotype switching.

### Transcriptional Regulation of CD40 Ligand Gene Expression

We have cloned the mouse gene for CD40 ligand, including 1.5 kb upstream of the transcription start site [6]. We have found several DNA elements that play an important role in CD40 ligand gene expression. Elements in the CD40 ligand promoter region show homology with elements in the IL-2 promoter region that include the nuclear factor of activated T cells.

### Conclusion

Studies with CD40-ligand-deficient humans and CD40 knockout mice indicate that CD40/CD40 ligand interactions are critical for T-cell-dependent isotype switching and for germinal center formation. CD40 does not seem to play a critical role in B cell development, and similarly CD40 ligand does not seem to play a critical role in T cell development although CD40 is expressed early in B cells, at the pre-B cell stage and CD40 ligand is expressed on single-positive CD4+ T cells. However, a role for CD40/CD40L interactions in thymic selection needs to be investigated.

38

Castigli/Fuleihan/Ramesh/Tsitsikov/ CD40 Ligand/CD40 Deficiency

Tsytsykova/Geha

### References

Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S: The CD40 antigen and its ligand. *Annu Rev Immunol* 1994;12:881-922.

Vercelli D, Geha RS: Regulation of isotype 4 switching. *Curr Opin Immunol* 1992;4:794-799.

Ramesh N, Fuleihan R, Geha RS: Molecular pathology of X-linked immunoglobulin deficiency with normal or elevated IgM (HIGMX-1). *Immunol* 1994;138:87-104. Fuleihan R, Ahern D,

Geha RS: Decreased expression of the ligand for CD40 in newborn lymphocytes. *Eur J Immunol* 1994;24:1925-1929.

Castigli E, Alt F, Bottaro A, Davidson L, Mizoguchi E, Bhan A, Geha RS: CD40 deficient mice generated by RAG-2 deficient blastocyst complementation. *Proc Nat Acad Sci USA*, in press.

Tsitsikov E, Ramesh N, Geha RS: Structure of the murine CD40 ligand gene. *Mol Immunol*, in press.

•

I

39