



Review

Expression and Function of CD30 on T Lymphocytes

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Abstract. T cell receptor, accessory molecules, cytokines are important regulatory factors that determine the development and function of T lymphocytes. Among them are also molecules belonging to superfamily of tumor necrosis factor receptor (TNFR) which beside CD30 include CD27, CD40, TNFR-I and -II, Fas (CD95), OX40, 4-1BB (CDw137), nerve growth factor receptor, lymphotoxin- β receptor, Apo3/DR3/Ws1-1/lymphocyte associated receptor of death, DR4, DR5/TNF-related apoptosis-inducing ligand, osteoprotegerin, and TNFR-related 2. CD30 recognized originally on Reed-Sternberg cells of Hodgkin's lymphoma became of interest in studies of Th1 and Th2 cell differentiation. This paper shows recent findings regarding CD30 expression and its pleiotropic role in T cell function. It provides information about controversial role of CD30 as Th2 cell differentiation marker and gives concise insight into the function of this receptor as a signal transducing molecule.

Key words: CD30; T cells; cytokines.

T lymphocytes play an important role in specific immune responses. Antigen recognition, local environment, the type of cells involved and genetic factors determine the generation of immunological responses. These responses may include processes leading to the development or selection of Th0, Th1, or Th2 cells which are distinguished based on the profile of cytokines they produce. Th0 lymphocytes which do not show apparent restrictions in regard to the production of the particular type of cytokine may develop into Th1 cells producing e. g. IL-2, INF- γ , TNF- β or into the Th2 subpopulation of cells producing IL-4, IL-5, IL-9, IL-10, IL-13^{28, 38, 40}. The existence of Th1 and Th2 subpopulations was originally defined by *in vitro* generated murine T cell clones²⁷ however, their presence among human T cells is still controversial. Our own studies and others^{37, 39} provide evidence that human T cells specific for "Th1" or "Th2" antigen show quantitative but not qualitative differences in regard to the expression of characteristic set of cytokines. The existence of phenotypically differentiated sub-

populations of Th1 and Th2 cells would be easier to determine by finding a specific marker. Studies of CD30 expression on T cells have suggested this receptor as a candidate for Th2 specific determinant. However, the role of CD30 has soon been found to be as controversial as whole division of human T cells into Th1 and Th2 subpopulations. Nonetheless these studies brought a new insight about the expression and the role of CD30 in T cell activity.

General Characteristic of CD30 Receptor

CD30 is a membrane glycoprotein of 120 kDa recognized originally by the mAb Ki-1 on Reed-Sternberg cells of Hodgkin's lymphoma¹³. Currently it is used as a clinical marker of this disease³⁶. CD30 belongs to the tumor necrosis factor receptor (TNFR) superfamily which includes also CD27, CD40, TNFR-I and -II, Fas (CD95), OX40, 4-1BB (CDw137), nerve growth factor receptor, lymphotoxin- β receptor, Apo3/DR3/Ws1-1/lymphocyte associated receptor of death, DR4, DR5/TNF-re-

lated apoptosis-inducing ligand, osteoprotegerin, and TNFR-related 2. The extracellular, N-terminal amino acid sequence of these proteins shows 25–35% homology and characteristic repeating cysteine-rich motifs. Cytoplasmic domains however show no homology at all¹⁸. Extracellular domain of CD30 contains binding sites for CD30 ligand (CD30L) while cytoplasmic domain binds TNFR associated factor (TRAF) proteins which play a crucial role in signal transduction^{1, 11}.

CD30 Expression on T Cells

CD30 receptor is expressed on CD4⁺, CD8⁺, and on virally transformed T cells. CD30 is activation dependent, present on CD45RO⁺ memory T cells² and accompanied by the p55 IL-2 receptor¹⁴. Only 0–2% of peripheral blood mononuclear cells isolated from healthy or atopic donors stain positive for CD30 receptor^{9, 22}. This generally accepted characteristics of CD30 expression does not however extend to its suggested, preferential presence on Th2 cells. Exclusive expression of CD30 on Th2 subpopulations is controversial and questioned by many scientists.

CD30 Expression on T Cells Different from Th2 Subpopulations

The fact that CD30 receptor is not a specific attribute of Th2 cell and that it is not exclusively expressed on any of T cell subpopulations identified based on cytokine profile synthesis is suggested by number of studies. CD30⁺ T cells can produce concomitantly IL-5 and Th1-dependent cytokine – IFN- γ ² which together with CD30 expression can be induced by IL-12^{3, 39}. The suggested existence of CD30, as a specific Th2 cell marker, contrasts also with finding it on *Mycobacterium tuberculosis*-stimulated cells and its dependence on the intracellular presence of IFN- γ ²⁹. Generally, not only CD30, but any stable, phenotypic differences between Th0, Th1, or Th2 cells have been reported^{5, 35}.

CD30 Expression on Th2 Lymphocytes

Exclusive expression of CD30 on Th2 subpopulations of T cells is suggested from *in vivo* as well *in vitro* studies. *In vivo* observations include studies of CD30 expression on cells obtained from patients suffering from “Th2” or “Th1”-dependent diseases. Significant number of T cells expressing CD30 is found in tissue samples obtained from those patients who suffer from Th2 activation dependent disease like: systemic sclerosis¹⁷, Omenns’ syndrom¹⁸, graft-versus-host dis-

ease¹⁹. No CD30 expression, on the other hand, is observed on cells obtained from patients suffering from “Th1” activation-dependent diseases and especially in those where high levels of IFN- γ are found. Among them are infections with *Helicobacter pylori* and Crohn’s disease¹⁹. The specificity of CD30 expression on Th2 cells is also suggested from number of studies concerning allergy and asthma. The number of CD30⁺ T cells obtained from bronchoalveolar lavage of asthmatic patients is high and contains about half of the $\gamma\delta$ T cell receptor (TCR) lymphocytes and 5–12% of $\alpha\beta$ TCR T cells³⁴. The number of these cells obtained from peripheral blood of allergic patients during the allergy season, although reported as very low or undetectable, increases after *in vitro* allergen stimulation. This increase correlates with symptomatic disease severity score and IL-4 levels. On the other hand, it inversely correlates with IFN- γ production and IFN- γ : IL-4 ratio^{9, 22, 23}. Furthermore, the *in vitro* established Th1 cell clones do not express CD30 mRNA, nor they express membrane or soluble form of this receptor, however, Th2 cell clones express substantial amounts of CD30 mRNA and its membrane form. They also release high quantities of soluble CD30⁹.

Regulation of CD30 Receptor Expression on T Cells

CD30 receptor is expressed on T cells upon their activation. Its presence on T cells is secondary to the TCR and/or accessory molecules mediated signals¹⁴. CD30 expression is missing from CD28 deficient, TCR transgenic murine T cells, as well as from CTLA-4 crosslinked wild-type T cells¹⁶ or antibody inhibited TNFR/related 2 (TR2)-another member of TNFR family²⁰. CD30 expression is also dependent on signals delivered by cytokines. The highest expression of CD30 on T cells is found between 48 and 72 h after an *in vitro* stimulation with anti-CD3 and rIL-2^{3, 14}. Among modulators of CD30 expression are also IL-12 and IL-4. Whereas IL-12 clearly upregulates CD30 expression^{3, 39} the role of IL-4 in this process is not clear. In murine model this cytokine is required for induction of CD30 on T cells during primary antigen stimulation and for maintaining it during secondary responses³¹. It has also been shown that IL-4 may substitute for CD28 in the upregulation of CD30 expression on CD28-deficient TCR transgenic T cells. Of interest, IL-13 which shares many functional characteristics with IL-4 did not mimic it in this process¹⁶. In contrast to these findings the addition of IL-4 to activated human peripheral blood mononuclear cells resulted in reduction of the number

of CD30⁺ T cells³. This discrepancy in IL-4 requirements for CD30 expression may be the result of general differences between human and murine T cells.

The Effect of CD30 Receptor Crosslinking on Development and Activity of T Cells

Although natural ligand for CD30 receptor is not known, the effect it exerts on T cells can be monitored by the use of agonistic antibodies¹⁰, CD30L transfected cells¹⁹, or fusion proteins consisting of sCD30 and Fc part of immunoglobulin (sCD30-Ig)¹⁰, and recently constructed fusion protein comprised of extracellular domain of human CD30L and extracellular domain of the human CD8 α chain (sCD30L/CD8 α)³². Crosslinking of CD30 receptor on human³ and murine T cells¹⁶ with agonistic antibodies provides co-signal for anti-CD30 induced primary and secondary proliferative responses. These responses may be indirectly affected by CD30-mediated increased production of IL-6, as has been shown for "T-cell like" Hodgkin's disease cells exposed to CD30L or stimulated with agonistic anti-CD30 antibody¹⁹. Agonistic antibodies against human CD30 receptor not only affect the proliferative responses but also determine the development of T cell populations. The blockade of CD30-CD30L interaction inhibits development of Th2 cells, lowers the production of IL-4 and IL-5 and shifts it toward Th1 profile¹⁰. Differentiated, Th0 clones and CD45RO⁺ T cells obtained from blood of normal subjects, on the other hand, show increased production of IFN- γ , IL-5², and to a smaller extent IL-4 after anti-CD30 antibody costimulation. Th2 clones in this way produce IL-5 and IL-4^{10, 25}.

CD30 receptor, although does not possess so-called death domain that is characteristic for CD95 or TNFR-I, it also contributes to cell death as has been shown by LEE et al.²¹ in the case of CD30⁺ T cell hybridomas or by POWELL et al.³² who used Karpas-299 and HDLM-2 cell lines. The pleiotropic effects of CD30 signaling were also reported by GRUSS et al.¹⁷. These effects varied depending on the type of the cell lines used and included such on opposite outcomes as increased proliferation, its reduction, or cytolytic cell death. Among other, known effects which are mediated by CD30 receptor are: translocation of NF- κ B transcription factor^{1, 19}, increase in intracellular calcium levels¹⁴ and induction of HIV gene expression in infected T cells²⁴.

CD30-Mediated Intracellular Signal Transduction

The pleiotropic effects of CD30 cross-linking are regulated by TRAF which bind to 2 independent cyto-

plasmic domains of this receptor^{7, 15}. Among TRAF signal transducing molecules which bind to CD30 cytoplasmic domains are TRAF1, TRAF2, TRAF3, and TRAF5^{1, 4}. Whereas TRAF1 and TRAF2 bind to both domains TRAF3 and TRAF5 binds only to domain located closer to the NH₂ terminal binding site. Interaction of TRAF1¹¹, TRAF2⁴ or TRAF5¹ with their binding sites within CD30 cytoplasmic domain activates NF- κ B transcription factor which regulates the transcription of several cytokines among which are: IL-2, IL-6, IL-8, IL-12, G-CSF, GM-CSF, and RANTES⁶. So far, the involvement of NF- κ B in CD30 induced cytokine synthesis was documented only for IL-6. There is however, no direct evidence for the role of TRAF signal transducing molecules in this process¹⁹. TRAF proteins are also involved in CD30-mediated apoptosis. Studies of TCR-dependent cell death of T cell hybridomas mediated by CD30 have suggested TRAF1 and TRAF2²¹ as likely candidates in this process. These suggestions were confirmed in studies which showed rapid depletion of TRAF2 nad TRAF1 during CD30 signaling and concomitant, increased sensitivity of lymphocytes to undergo apoptosis through e.g. TNFR1-mediated apoptotic signals¹².

Conclusions

CD30 is a membrane glycoprotein which belongs to the tumor necrosis factor receptor superfamily. Its expression is activation dependent, present on CD4⁺, CD8⁺, CD45RO⁺, memory T cells which co-express p55 IL-2 receptor. Its role as a Th2 specific marker is controversial. CD30 mediates pleiotropic effects including increased antigen-mediated cell proliferation, translocation of NF- κ B transcription factor, induction of HIV expression by infected T cells, and cell death. Some of these effects are mediated by TRAFs. CD30 receptor mediates increased expression of IL-6 through NF- κ B induction. However, the way in which CD30 induces or upregulates expression of IL-4, IL-5, and IFN- γ is not explained yet. It may however, suggest that transcription factors other than NF- κ B are involved in CD30-mediated signaling since NF- κ B has not been shown to regulate the expression of IL-4, IL-5, and IFN- γ . Even though CD30 expression and function is secondary to the signals mediated by TCR or CD28, CTLA-4, through its pleiotropic effects this receptor may be considered as a very important regulator of T cell responses.

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