

Division of Immune Receptors and T Cell Activation

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We are interested in the contribution of cell surface molecules (positive and negative costimulatory molecules) on human T cells and antigen-presenting cells (APC) in immune responses. For our studies we have developed experimental systems that make it possible to efficiently analyse the contribution of individual ligands to T cell activation processes. In addition we are trying to identify novel receptors that regulate the activation of T cells by APC. To achieve this goal we rely on our experience in the generation, expression and screening of cDNA expression libraries. Another focus of our research is the identification of cell surface antigens that are recognized by sera derived from cancer patients, transplant recipients or from stem cell transplanted individuals. For these projects we are developing efficient methodologies to screen retroviral expression libraries with polyclonal antibody preparations like human sera.

The role of individual costimulatory molecules in the activation of human T cells

A large number of positive and negative costimulatory molecules govern the interaction of antigen-presenting cells with T cells (Figure 1a). The manipulation of such pathways offers attractive avenues to enhance immunity to pathogens but also to attenuate unwanted responses in autoimmune conditions or transplantation. While the role of most costimulatory molecules has been extensively studied in animal models, functional data on the effects of several of these molecules on human T cells are much more limited. It is evident that a complete understanding of the role of human costimulatory molecules is a prerequisite to identify good therapeutic targets.

We are trying to learn about the contribution of individual receptor-ligand pairs to T cell activation processes and also to identify novel molecules that play a role in this context. We are studying the consequences of costimulatory signals on primary human T cells by assessing classical parameters like proliferation, cytokine secretion and activation-marker regulation. In addition, we are using genome-wide gene expression analysis and biochemical assays on intracellular signalling processes to get a more complete picture on the function of costimulatory signals during the activation of human T cells.

For our studies we have developed a novel system of T cell stimulators. This system is based on the murine Bw5147 cell line that was engineered to express a membrane-bound anti-human CD3 single chain antibody. By ligating their T cell receptor complex, this antibody gives "Signal 1" to human T cells. Upon expression of costimulatory molecules on our stimulator cells, their role in T cell activation processes can readily be studied (Figure 1b). We find this system to be an excellent tool to study the role of individual costimulatory molecules in their natural conformation but detached from the complex surface of an APC that harbours a plethora of costimulators.

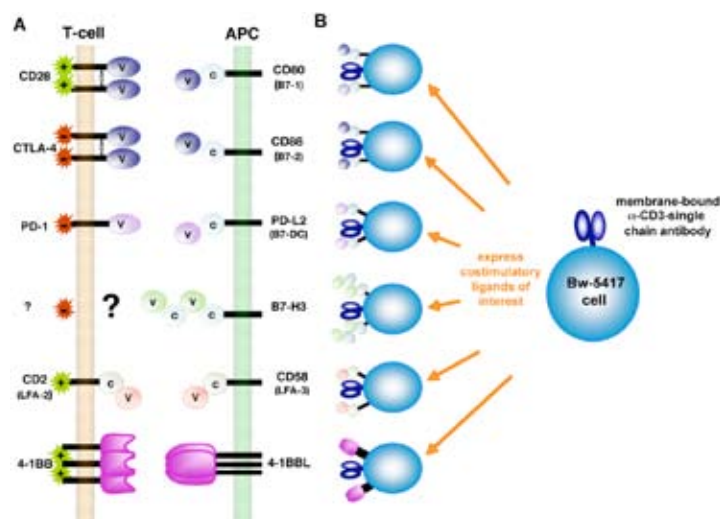


Fig. 1:

A) Selected costimulatory ligands that regulate T cell activation processes by interacting with their activating or inhibitory receptors on T cells.

B) Our system of T cell stimulators is a straightforward and versatile system to study the contribution of individual costimulatory ligands to the activation of human T cells.

Although our current studies focus on members of the B7- and the TNF-superfamilies we have generated a large set of T cell stimulator cell lines expressing additional human costimulatory molecules. Thus we are currently in the unique position to test and compare virtually all human molecules implicated in T cell costimulatory processes in one experimental system. We have recently used this system to analyze the T cell stimulatory capacity of TNF family members. In this study we show that under conditions where 4-1BBL, OX40L, CD70 and GITRL readily costimulated human T cells, CD30L and LIGHT consistently failed to contribute to T cell activation processes, indicating that these molecules might be functionally distinct from the costimulatory members of this family (Kober et al. 2009).

We are currently investigating the interplay between T cell costimulatory signals and immunomodulatory or immunosuppressive drugs. Our preliminary results indicate that the quality and strength of the costimulatory signals greatly affects the capacity of such drugs to inhibit T cell responses (Figure 2). We believe that a better understanding of the interplay between immunosuppressive drugs and costimulatory signals might eventually lead to improved therapeutic strategies to downmodulate unwanted T cell responses.

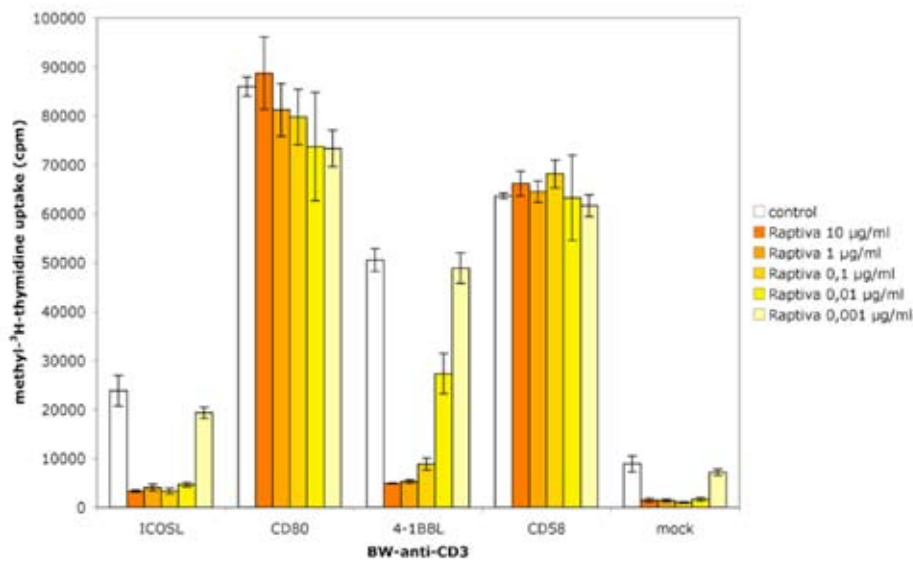


Fig. 2: Strong costimulatory signals exerted by CD58 and CD80 completely abrogate the ability of the therapeutic CD11a-Antagonist Raptiva (Efalizumab) to inhibit the activation of human T cells. Primary human T cells were activated with human T cell stimulator cells expressing costimulatory ligands in the presence of indicated concentrations of Raptiva.

Loss of the costimulatory receptor CD28 is an important phenotypic correlate for senescent T lymphocytes in humans. Such cells are functionally impaired and contribute to immune dysfunction in elderly and chronically infected individuals. Since CD28 negative T cells are deficient in the primary costimulatory pathway, insufficient activating signals might contribute to the functional senescence in this cell population. We are therefore analysing whether signals from alternative costimulatory molecules are able to revert the dysfunctional state of CD28 negative T cells derived from elderly individuals. This project is carried out in close collaboration with the group of Prof. Grubeck-Loebenstein, Institute of Biomedical Aging Research of the ÖAW, Innsbruck, Austria.

In addition we also generate and use immunoglobulin fusion proteins representing the extracellular domains of costimulatory molecules to study their functional role as well as the interaction of costimulatory ligands with their receptors. There is considerable controversy regarding the role of the B7-family member B7-H3 during the activation of T cells since inhibitory as well as activating functions have been ascribed to this molecule. Using fusion proteins representing the extracellular domains of the B7-family member B7-H3, we recently have been able to show that B7-H3 consistently and potently inhibits T cell activation. Furthermore by performing extensive binding experiments with fusion proteins representing B7-H3 and TREML-2, we have been able to show that TREML-2, which was recently claimed to serve as a costimulatory B7-H3 receptor does not interact with B7-H3 (Leitner et al.2009). Thus B7-H3 is an orphan ligand and we are using our B7-H3-immunoglobulin fusion proteins in conjunction with retroviral expression cloning to identify receptors, which mediate the potent T cell inhibitory effects of B7-H3.

Identification and characterization of surface antigens that elicit antibody responses in tumour patients or in individuals transplanted with solid organs or hematopoietic stem cells.

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for hematological and oncological diseases. Immune response to residual malignant cells (Graft-versus-leukemia; GvL) can contribute to the eradication of the tumours in the transplant recipients. However, graft-versus-host disease (GvHD) is a frequent complication of HSCT, contributing to morbidity and mortality. An important mechanism underlying both effects is the recognition of antigens by the donor derived immune cells. To date most efforts to search for molecules recognized following allogeneic HSCT have focused on the identification of antigens recognized by T cells. However antibodies directed to membrane resident antigens might also play an important role since they might directly eliminate cells by antibody- or complement dependent cytotoxicity. However, efficient methodologies to define such antigens have not been described to date.

Using retroviral expression cloning we have recently identified the surface molecule immunoglobulin transcript 5 (ILT-5) as a polymorphic antigen that is able to elicit potent immune responses in over 5 % of HSCT recipients (Pfistershammer et al. 2009). We demonstrated that ILT5 antibodies efficiently kill ILT5 bearing cells and furthermore observed a very strong increase in the ILT5 antibody titres following leukemic relapse (Figure 3). These results represent the first description of potent allogeneic antibody responses to a non-MHC surface molecule following HSCT and indicate that humoral immune responses might contribute to GvL and GvHD responses.

Our results provide evidence that allogeneic surface antigens that are recognized in HSCT recipients can be identified using eukaryotic expression cloning. In close collaboration with the group of Prof. Greinix at the Bone Marrow Transplantation Unit of the MUW and other centres where HSCT is performed, we have established a large collection of sera from HSCT patients that we plan to use for the identification of additional antigens. Beneficial anti-tumour immunity as well as deleterious immune responses against allogeneic solid organ grafts are of significant clinical relevance. Consequently they have been subjects of intense research during the last decades. In these studies the focus has been on intracellular antigens as well. In close collaboration with Dr. Pfistershammer, who is now at the Department of Dermatology, we have initiated studies to identify non-HLA surface antigens recognized by tumour patients and recipients of renal grafts. Also in these studies we rely on our extensive experience in the screening of eukaryotic cDNA expression libraries with patients' sera. For these projects we collaborate with clinicians who provide us with patients' sera and clinical information.

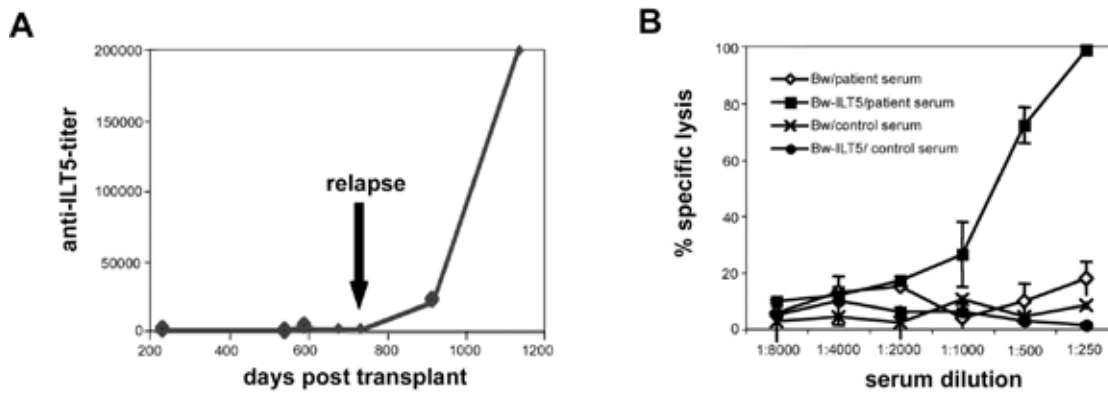


Fig. 3: Humoral immune responses to the polymorphic immunoglobulin-like transcript 5 (ILT5) in hematopoietic stem cell transplant recipients. A) ILT5-specific antibodies strongly increase in a patient following relapse from AML. B) ILT5-reactive sera efficiently kill ILT5 bearing cells by complement-dependent cytotoxicity.

Anti-thymocyte globulins (ATGs) are polyclonal antibody preparations that are widely used as very effective immunosuppressive agents. Recent research has demonstrated that ATGs can exert potent and broad immunomodulatory functions by interacting with different types of T cells including regulatory T cells and with dendritic cells. Currently there is limited information regarding the cellular targets of ATG, which are responsible for their broad immunomodulatory and immunosuppressive effects. By screening an eukaryotic expression library derived from human dendritic cells with ATGs we have been able to identify several DC antigens that are strongly recognized by ATG antibodies. We will employ eukaryotic expression libraries derived from freshly prepared and in vitro activated human PBMC to identify the ATG specificities that are directed to surface molecules expressed on these cells.

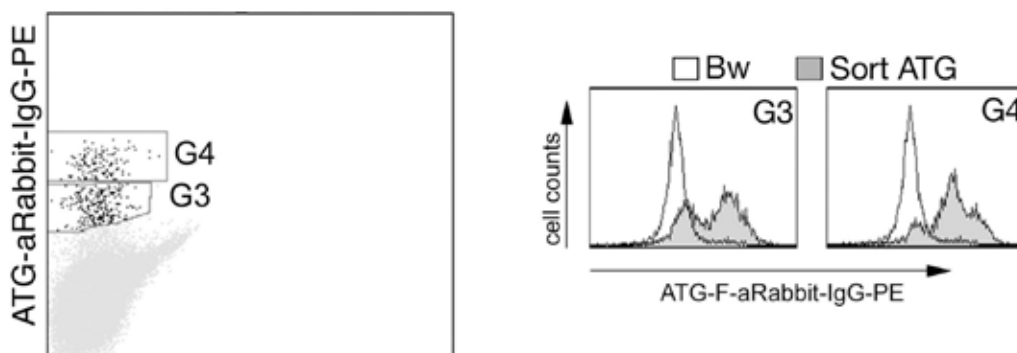


Fig. 4 Screening of a retroviral cDNA-expression library derived from human Dendritic cells with ATGs. Left panel: A cell pool representing the cDNA library was incubated with ATG and cells expressing ATG antigens (Gates 3 and 4) were isolated by FACS. Right panels: The isolated cell pools react with ATG.

Grants

- OeNB (Österreichische Nationalbank) "Responsiveness of CD28-negative T cells to alternative costimulatory signals" 1.1.2008-31.05.2010.
- ÖAW (Austrian Academy of Sciences) Doc Forte fellowship "Identification of tumor antigens using antibodies induced by DC-based vaccines" 1.1.2009 - 31.12.2010 (to Judith Leitner)
- EKFS (Else Kröner Fresenius Stiftung) "Defining the major specificities in ATGs" 1.9.2009-29.02.2012
Bürgermeisterfonds 09051 "Interaktion von Antithymozytenglobulin (ATG) mit dendritischen Zellen" 2009-2011

Theses**Diploma Theses**

- Ramona Woitek: Costimulatory molecules and exogenous cytokines in the long-term expansion of human T-cells, 01.03.2006 - 1.10.2007
- Werner Kuschei: Effects of TNF and CD11a antagonists on T cell costimulatory pathways. Since 1.10.2008

Master Thesis

- Karin Drobits: The interplay between immunosuppressive drug and costimulatory signals in the activation of human T cells. Since 01.08.2009.

PhD Thesis

- Judith Leitner: Identification of tumor antigens using antibodies induced by DC-based vaccines. Since 01.08.2006

Awards

- Judith Leitner: Doc Forte Fellowship awarded 11.2008

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3. Pfistershammer K., C.Klauser, J.Leitner, J.Stöckl, O.Majdic, T.Weichhart, Y.Sobanov, V.Bochkov, M.Säemann, G.Zlabinger, P.Steinberger. Identification of the scavenger receptors SREC-I, Cla-1 (SR-BI), and SR-AI as cellular receptors for Tamm-Horsfall protein. *J.Leukocyte Biology* 83:131-138 (2008)
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5. Pfistershammer, K., A.Lawitschka, C.Klauser, J.Leitner, R.Weigl, M.H.Heemskerk, W.F.Pickl, O.Majdic, G.A.Böhmig, G.F.Fischer, H.T.Greinix, P.Steinberger. Allogeneic disparities in immunoglobuline-like transcript 5 (ILT5) induce potent antibody responses in hematopoietic stem cell transplant recipients. *Blood*, in press (2009)