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Session 7: Immunomodulation

Thursday 13th November 2008. Moderator: Mark Glassy

[14.00–14.30]
Antibodies at the cross-roads of regenerative medicine
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Abstracts not provided.

[14.30–14.50]
Tolerogenic properties of anti-alpha/beta T Cell receptor monoclonal antibody in face transplant model
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Purpose: Development of microsurgical techniques allowed introducing composite tissue allografts (CTAs) into the armamentarium of plastic and reconstructive surgery. In experimental model of face transplants we achieved operational tolerance in fully MHC mismatched rat model under cyclosporine-A (CsA) monotherapy. In this study we tested the effect of immunotherapy with donor bone marrow transplantation (DBMT) under selective immunodepletive protocol using anti-alpha/betaTCRmAb and CsA in face allograft model.

Methods: Thirty six hemiface allotransplantations were performed between LBN(RT1^{l+n}) donors and LEW(RT1^{l}) recipients in 6 groups (6 rats each). Controls: Group 1 isograft and Group 2 allograft without treatment, Group 3 received intraosseous DBMT only. Groups 4, 5 and 6 received 7-day immunodepletive protocol of anti-alpha/betaTCRmAb/CsA. Groups 5 and 6 were augmented with intraosseous DBMT of 35×10^6, and 100×10^6 cells respectively. Before transplantation bone marrow cells (BMC) were stained with PKH dye to evaluate engraftment and migration of donor BMC.

Flow cytometry assessed immunodepletion of T–lymphocytes, donor-specific chimerism for MHC class I (RT1^{n}) antigens and presence of regulatory T-cells CD4+/CD25+.

Results: Isograft survived indefinitely, controls without immunosuppression rejected allograft within 5 to 8 days. Group 3 with DBMT only accepted transplant up to 13 days. Median survival time (MST) of facial allograft under alpha-betaTCR/CsA therapy (Group 4) was 35 days. In both Groups 5 and 6 augmented with DBMT MST was 48 days. However, the longest survival time 465 and 498 days was in Groups receiving 35x10^{6}or 100x10^6 BMC respectively. In long-term survivals split tolerance was found, recipients accepted skin and rejected hair components.

PKH-positive cells of donor origin were present within lymphoid organs and skin of recipients. In rejected allografts T-cell chimerism declined to $<$1%, and B-cell chimerism was at 4.5% of CD45RA/RT1^{n}. In contrast, in long-term survivals T-cell chimerism was 2%-4% (RT1^{n}) during follow-up period and B-cell chimerism reached 12.4% CD45RA/RT1^{n} at day 465 post-transplant. Regulatory T-cells CD4+/CD25+ were detected at 4.0% vs 2.2% in long-term survivals vs rejected allografts respectively.

Conclusion: Long-term face allograft survival was achieved under 7-day alpha/beta-TCRmAb/CsA protocol augmented with DBMT, without chronic immunosuppression, and was associated with the presence of regulatory T-cells and maintenance of donor T-cell and B-cell chimerism.

[14.50–15.10]
Alteration of HIV gp120 antigenicity and immunogenicity by antibodies to the CD4-binding site of gp120
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HIV envelope gp120 is a crucial antigenic target for Ab responses against the virus, but most, if not all, of the critical gp120 epitopes are either not readily accessible for Ab recognition or are poorly immunogenic for eliciting Ab response. Since antibodies are known for their capacity to alter immune responses to the specific antigens, we investigated the use of immune complexes as immunogens to improve the overall Ab titers against gp120 as well as to target the Ab response toward specific neutralizing epitopes. Specifically, we tested the immune complexes made of gp120 and human mAbs to the CD4-binding site (CD4bs) of gp120 because these mAbs display several unique properties. The anti-CD4bs mAb binding induces significant structural changes in gp120 that may potentially expose specific epitopes on gp120. The gp120/anti-CD4bs complexes are also more resistant to proteases and other degradative enzymes and may serve as a durable antigen source for stimulating gp120-specific B cells and Ab production. Hence, we evaluated the antigenicity and immunogenicity of gp120 when complexed with human anti-CD4bs mAbs. The data show that gp120 bound by the anti-CD4bs mAbs had higher reactivity with Abs to specific regions of gp120, including the neutralizing epitopes in the V3 loop. Immunization of mice with these complexes also elicited higher titers of gp120-specific serum IgG and IgA than immunization with uncomplexed gp120 or other gp120/mAb complexes. Notably, the enhanced Ab production was directed against V3 and correlated with better exposure of this region on the gp120/anti-CD4bs mAb complexes. Moreover, potent virus-neutralizing activity was observed in the sera from mice immunized with the gp120/anti-CD4bs mAb complexes, although the breath of neutralization was narrow. Additional studies are in progress to test various gp120/mAb combinations and different immunization parameters to broaden the breath of neutralizing Ab responses. Overall, these results indicate that the use of immune complexes may be a promising approach to improve the immunogenicity of HIV envelope and to direct the Ab responses toward neutralizing epitopes on this antigen.

[15.10–15.40]
Manipulation of T cell populations with antibody/mAb complexes

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Cytokines are low molecular weight molecules that control development, survival and function of lymphocytes. For T cells, the most relevant cytokines are those that interact with the receptors (R) expressing the common gamma chain (γc): IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. IL-2 is a prototypic T cell cytokine produced mainly by activated T cells and, in autocrine and paracrine fashion, supports survival, proliferation and effector function of typical activated $\alpha\beta$ T cells. IL-2 is also an essential survival factor for regulatory T cells (Tregs) that exists to suppress excessive T cell responses. IL-7 and IL-15 are produced by non-T cells and are vital for the development of T cells and NK cells, respectively. These two cytokines are also indispensable for controlling homeostasis of naïve and memory T cells. Thus, mature T cells fail to survive or undergo homeostatic proliferation in the absence of these two cytokines.

Despite the ever-increasing understanding of the biology of the γc family of cytokines, the therapeutic potential of these cytokines has yet to be realized, and, especially for IL-2, is fraught with strong side effects. Here, a key problem is that how cytokines are presented or recognized under in vivo conditions is still largely obscure. Past studies showed that the half-life of cytokines in vivo could be extended by binding to a monoclonal antibody (mAb) specific for the cytokine. We have recently re-visited this phenomenon and showed that, for several γc cytokines, cytokine/anti-cytokine mAb complexes dramatically increase the biological activity of cytokines for T cells under in vivo conditions. Typically, cytokines administered as complexes displayed100-fold higher activity than free cytokines. This applied to IL-2, IL-4, IL-7 and IL-15; for IL-15, the complexes were generated with its naturally presenting IL-15Rα chain.

Interestingly, for IL-2, depending on the particular mAb used, the IL-2/mAb complexes preferentially induce either strong proliferation of Tregs or memory CD8 plus NK cells; functionally, this translates into mediating either immune suppression or augmentation. The selective activity of the two types of IL-2/mAb complexes appears to reflect preferential reactivity towards two different forms of IL-2R. Specifically, one complex interacts with the trimeric form of

IL-2R, which is expressed by Tregs and recently activated T cells, while the other complex binds to the dimeric form of IL-2R expressed on memory CD8 and NK cells.

Exactly why cytokine/mAb complexes are so much more potent than free cytokines is not known, but this is an in vivo phenomenon, as increased activity is not observed under in vitro conditions. Despite this gap in knowledge on their mechanism high activity, the cytokine/mAb complexes appear to have a great clinical potential for modulating immune responses.