Old Herborn University Seminar Monograph 16:
Host microflora crosstalk.
Editors: Peter J. Heidt, Tore Midtvedt, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 91-93 (2003).

HOW MUCOSAL IMMUNITY IS CONTROLLED BY LOCAL FACTORS

STEFAN C. MEUER

Institute for Immunology, Ruprecht-Karls-University, Heidelberg, Germany

The mucosal immune system, the body's largest immunological compartment, is constantly exposed to an enormous load of foreign antigens derived from commensal bacteria and food. Under physiologic circumstances, however, no systemic immunity can be generated at this interphase of the body with its environment. Given that the T lymphocyte population which exists in the lamina propria and organised tissues of the mucosa (LPT) is of polyclonal nature one has to assume that luminal T cells can interact with antigenic determinants present in this location. Under physiologic circumstances, however, "typical" immune responses are not generated. Since such reactions of the mucosal immune system toward luminal antigens result in inflammatory reactions as observed in various types of inflammatory bowel diseases.

An initial important clue to understanding why mucosal T cells, unlike peripheral blood or lymphnode T cells, do not respond to antigen encounter by their specific T cell receptors with systemic immune responses *in vivo* was the finding that freshly isolated LPT when compared with peripheral blood T cells (PBT) do not undergo clonal expansion/proliferation and cytokine production when stimulated *in vitro* through their CD3 antigen-receptor complex. Interestingly, no phenotypic abnormalities could be detected in LPT when analysed extensively in order to understand their lack of proliferation to T cell receptor simulation. A second set of experiments then made obvious that this fundamental observation could not be explained at the level of T cells: When LPT were mixed with peripheral blood macrophages and subsequently simulated through TCR/CD3 their proliferative behaviour was comparable to that of PBT. In a reciprocal fashion, PBT when incubated with mucosal macrophages could not respond to such stimulation. Therefore, one had to conclude that central regulators of immune responses in the gut where derived from the monocyte/macrophage lineage. A phenotypic comparison between mucosal monocytes and monocytes circulating in peripheral blood then pointed towards substantial differences between these two cell-populations in that the receptor for LPS (CD14) as well as a variety of additional adhesion molecules known to be required for T cell co-stimulation such as CD58 and CD54 were down-regulated on LPMO. The functional consequences of this phenotypic alteration and the question how such a particular phenotype as observed in LPMO can be generated will be dealt with below.

Earlier data had indicated that the mucosal environment produces pro-oxidative substances of as yet unknown nature. Thus, supernatants generated from mucosal cells (epithelial cells and monocytes) when added to peripheral blood mononuclear cells (PBMC) dose dependently inhibited their proliferative response to antigen receptor stimulation. If one, however, added reducing substances such as 2-mercapo-ethanol (2-ME) inhibition was abolished and proliferation was normal. This suggested that the activity of pro-oxidative

locally secreted products could be counteracted by reducing agents and pointed towards the direction of a physiologic pro-oxidative state of the mucosal microenvironment. This finding was of particular interest because T lymphocytes are considerably sensitive to inhibition by pro-oxidative substances. This is due to the fact that they do not express the cystin/glutamate transporter complex in their plasma membranes. Thus, therefore, they cannot take up cystin which represents the precursor for the synthesis of Glutathion (GSH), one of the most potent intracellular reducing systems. Regular GHS-levels are required for cell-cycle progression, transcriptional activity and likely also for the stability of intracellular proteins (particularly those which exist as disulphide linked dimers). Given the above finding of a pro-oxidative state it was important to investigate the possibility whether GSH-levels in LPT would be significantly lower than in PBT which was indeed the case. Thus, LPT contain only 10% - 20% of the GSH concentrations as PBT. Needless to say, the availability of reducing substances leads to an increase in intracellular GSH-concentrations in LPT with an alteration of their functional phenotype e.g. with regard to proliferation.

Physiologically, antigen encounter by T cells only leads to clonal T cell expansion and an immune response specifically directed at antigen when antigens are presented on appropriate MHC molecules and when appropriate additional stimuli are provided towards them (co-stimulation).

Given that T cells lack expression of the cystin-transporter (see above) they are dependent for their GSH-synthesis pathway on cells which can produce a secrete cysteine in their vicinity. This is an important activity and contribution of monocytes to T cell responses. Comparative analysis of monocytes from blood and monocytes from the mucosa clearly demonstrated that mucosal monocytes, unlike their blood counterparts cannot produce cystein as a co-factor for T cell activation. Importantly, cystein production by blood monocytes does not occur spontaneously but has to be induced through engagement of particular cell-surface-receptors including CD14 (LTS-receptor) and CD58 (LFA-3) which are exactly those that are not expressed but LP/MO.

It was now very important to elucidate the mechanism how cell-surface-receptors such as CD14 or CD58, respectively, can be down-regulated on monocytes. There exists one cytokine known to exert such an effect, namely interleukin 10 (IL-10). It was to our surprise to discover that IL-10 is produced in large quantities by mucosal epithelial cells, which are known to secrete their products largely in a basolateral direction, i.e. towards the lamina propria. There, IL-10 could potentially down-regulate the above mentioned cell surface receptors with the consequence that monocytes can no longer be induced to produce cystein.

Having worked up our way from the T cell as the most distal effector element in an interactive compartment we identified mucosal monocytes as central regulators of T cell reactivity. The latter, however, are controlled by mucosal factors such as pro-oxidative substances and epithelial cell derived interleukin 10.

Most probably, activities of epithelial cells require induction or stimulation by luminal components. The intestinal microflora through interaction with epithelial cell components may play an important role for the physiologic function of the latter, which dictate the functional programs of immunocompetent cells homing to the lamina propria. Therefore, it will be of interest for an understanding of homeostasis in the largest

immunological compartment of the human body to elucidate precisely the mechanisms through which - in a symbiotic fashion - commensal flora influences functional phenotypes of the body's cellular elements.

In conclusion, the presented studies of mucosal immunity highlight aspects of physiological and pathological immune regulation and point to the direction that therapies of mucosal inflammatory processes which target immunocompetent cells are, despite ameliorating acute symptoms are unlikely to affect the causal processes underlying unwanted immune responses. The latter rather result as a consequence of a disturbed microenvironment, which is characterised by epithelial, cells interacting with microbial components. Therefore, eventually, therapies which are directed at those elements will address pathologic processes at their basis.

THE AUTHOR'S LITERATURE CONCERNING THIS FIELD

Pirzer, U., Schürmann, G., Post, S., Betzler, M., and Meuer, S.C.: Differential Responsiveness to CD3-Ti versus CD2-dependent activation of human intestinal T lymphocytes. Eur. J. Immunol. 20, 2339-2342 (1990).

Qiao, L., Schürmann, G., Betzler, M., and Meuer, S.C.: Activation and signalling status of human lamina propria lymphocytes. Gastroenterology 101, 1529-1536 (1991).

Qiao, L., Schürmann, G., Betzler, M., and Meuer, S.C.: Down-regulation of protein kinase C activation in human lamina propria T lymphocytes: Influence of intestinal mucosa on T cell reactivity. Eur. J. Immunol. 21, 2385-2389 (1991).

Schürmann, G., Betzler, M., Post, S., Herfarth, Ch., and Meuer, S.C.: Soluble interleukin-2 receptor, interleukin-6 and interleukin-l in patients with Crohn's disease and ulcerative colitis: Preoperative levels and postoperative changes of serum concentrations. Digestion 51, 51-59 (1992).

Qiao, L., Schürmann, G., Autschbach, F., Wallich, R., and Meuer, S.C.: Human intestinal mucosa alters T cell reactivities. Gastroenterology 105, 814-819 (1993).

Qiao, L., Golling, M., Autschbach, F., Schürmann, G., and Meuer, S.C.: T cell receptor repertoire and functional behavior of lamina propria T lymphocytes in inflammatory bowel disease. Clin. Exp.Immunol. 97, 303-308 (1994).

Autschbach, F., Schürmann, G., Qiao, L., Merz, H., Wallich, R., and Meuer, S.C.: Cytokine messenger RNA expression and proliferation status of intestinal mononuclear cells in noninflamed gut and Crohn's disease. Virchows Archiv B 426, 51-60 (1995).

Qiao, L., Braunstein, J., Golling, M., Schürmann, G., Autschbach, F., Möller, P., and Meuer, S.: Differential regulation of human T cell responsiveness by mucosal versus blood monocytes. Eur. J. Immunol. 26, 922-927 (1996).

Braunstein, J., Qiao, L., Autschbach, F., Schürmann, G., and Meuer, S.: T cells of the human intestinal lamina propria are high producers of interleukin 10. Gut 41, 215-220 (1997).

Autschbach, F., Braunstein, J., Helmke, B., Zuna, I., Schürmann, G., Niemir, Z., Wallich, R., Otto, H.F., and Meuer, S.C.: *In situ* expression of intereukin-10 in noninflamed human gut and in inflammatory bowel disease. Am. J. Pathol. 153, 121-130 (1998).

Stallmach, A., Wittig, B., Giese, T., Pfister, K., Hoffmann, J.C., Bulfone-Paus, S., Kunzendorf, U., Meuer, S.C., and Zeitz, M.: Protection of TNBS-induced colitis by an IL-2IgG2b fusion protein in mice. Gastroenterology 117, 866-876 (1999).

Sido, B., Braunstein, J., Breitkreuz, R., Herfarth, C., and Meuer, S.C.: Thiol-mediated redox regulation of intestinal lamina propria T lymphocytes. J. Exp. Med. 192, 907-912 (2000).