# THE BV8/ PROKINETICIN SYSTEM AS A NOVEL MODULATORY FACTOR OF THE IMMUNE SYSTEM

Franchi Silvia, Panerai Alberto, Negri Lucia[1], Melchiorri Pietro[1] and Sacerdote Paola

Department of Pharmacology, University of Milan, via Vanvitelli 32, 20129 Milano, Italy.
[1] Department of Pharmacology and Human Physiology, Università La Sapienza, piazza Aldo Moro, Roma

Bv8, a 77 aminoacid protein recently isolated from *Bombina variegata* skin, belongs to a novel family of secreted proteins whose homologues have been described in rodents and human (prokineticins). Two G-coupled receptor named PKR-1 and PKR-2 have been found for Bv8. The biological activities linked to Bv8/Prokineticin are rapidly increasing; in particular a hyperalgesic activity of Bv8 has been demonstrated. There is now evidence that Interleukin (IL-) 1 and Tumor necrosis factor alpha (TNF-α) are involved in the creation of inflammatory pain; while the anti-inflammatory cytokines limited there. Considering that lymphoid organs, circulating leukocytes and hematopoietic cells express high levels of Bv8-like proteins as well as of their receptors we consider immune cells as a possible target for Bv8. In this study we test the impact of Bv8 administration on several mouse macrophage and T cell functions. We investigate, through the use of KO mice, the receptor subtype involved in the observed effects and we try to explain the mechanism by which Bv8 acts. After RT-PCR identification of PK2 and of its receptors on mouse peritoneal macrophages we demonstrated the induction of a pro-inflammatory phenotype of these cells by Bv8 both in vivo and in vitro: the protein stimulated macrophage migration and increased the production of the pro-inflammatory cytokines IL-1 and IL-12, decreasing that of the anti-inflammatory IL-10. Experiments with cells from PKR-1 KO mice demonstrated the involvement of this receptor which is probably coupled to a Gq protein as suggested by our study in which the effects of Bv8 on chemotaxis and cytokines were not PTX sensitive while they were completely prevented by the phospholipase C inhibitor U73122.
Considering the Bv8 effect on IL-12 and IL-10, the critical factors for the development of a correct Thelper (Th)-1/Th2 subsets, we could hypothesise an impact on Th-1/Th-2 balance. We checked the Bv8 effect (in vitro/ in vivo) on mouse splenocyte cytokines both in presence of the policlonal mitogen Concanavalin-A and after immunization of mice with the protein antigen keyhole-limpet hemocyanin (KLH). Our results indicated that the presence of Bv8 significantly affected the production of Th1/Th2 cytokines: we observed a significant decrement of the Th2 cytokines IL-4 and IL-10, while the Th1 cytokines IL-2 and IFNγ were not modified. These effects were lost in PKR-1 KO mice. In conclusion we demonstrated that Bv8 is a potent modulatory factor of the immune responses, and suggests that the prokineticin system can be a new target for inflammatory diseases.