Supplemental online material for

"Immunomodulatory mast cells: Negative, as well as positive, regulators of innate and acquired immunity",

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Figure legends 1-3 with references.

Supplemental Figure 1 with legend

Figure 1. Mast cell and basophil development and tissue distribution during examples of immune responses. Tissue mast cells are derived from hematopoietic stem cells (HSCs), which ultimately give rise to mast-cell progenitors (MCP). MCPs circulate in the blood, enter the tissues and there undergo differentiation and maturation, becoming mature mast cells whose phenotype can vary depending on the growth factor milieu (SCF, IL-3, etc.) and other microenvironmental factors. For example, so-called “mucosal mast cells” are found in the mucosa of the gut while “connective tissue mast cells” reside in the submucosa and muscularis propria. The numbers of these mast-cell populations can increase dramatically during a T_H2 response to parasitic infection of the gut[1]. Basophils, which develop in a pathway different from that of mast cells, leave the bone marrow in mature form and are then recruited into the tissues; numbers of basophils can increase markedly, in response to IL-3, e.g., during parasitic infection[2].

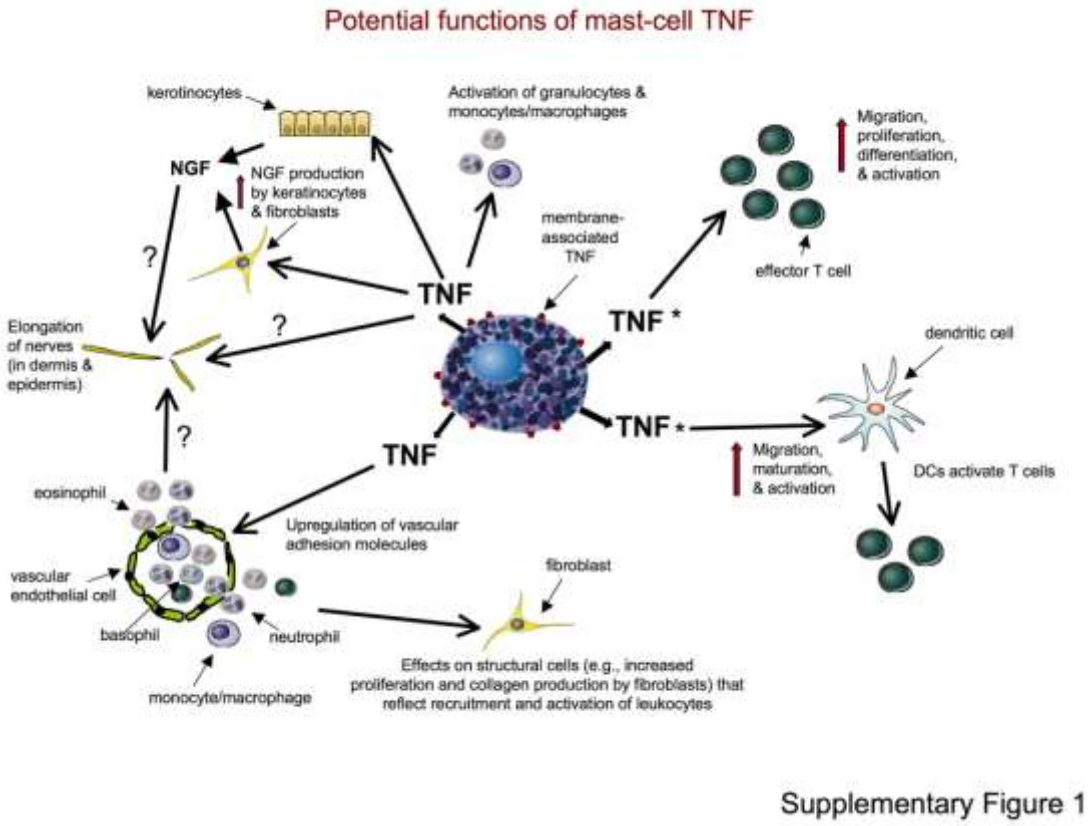
Figure 2. Potential functions of mast-cell IL-10. IL-10 can directly inhibit production of prostanoids by neutrophils[3] and pro-inflammatory cytokines by macrophages[4]. By directly inhibiting keratinocyte TNF and IL-6 production[5], IL-10 can indirectly reduce expression of adhesion molecules on vascular endothelial cells and thereby diminish the recruitment of circulating effector cells. IL-10 can directly promote the development of Tr1 and IL-10-secreting regulatory T cells (T_{Reg})[6,7] and also can enhance the ability of DCs[7,8] to reduce T-cell proliferation and cytokine production. While many of the specific functions indicated are

based on evidence from *in vitro* studies, mast-cell-derived IL-10[9] has been shown to mediate negative immunomodulatory functions *in vivo*.

Figure 3. **Hypothetical model of how mast cells might promote (in CHS response 1 [A-D]) or limit (in CHS response 2 [E-H]) features of CHS responses to haptens. (A-B)** Mast cells promote migration of DCs from the site of cutaneous sensitization by epicutaneous application of hapten (e.g., with 2% Ox in 100% ethanol)[10]. (**C-D**) 1 d after epicutaneous challenge with 1% Ox in 100% ethanol (i.e., day 6 after sensitization) mast cells promote features of the response, in part by directly or indirectly undergoing antigen- (Ag-) dependent activation and releasing mediators (including TNF) which, in that context, have net "pro-inflammatory" effects[10]. "?": The mechanism of mast-cell activation in this context is not yet clear, but may involve low levels of Ag-specific IgE and/or other mechanisms. (**E-F**) 1-2 d after epicutaneous challenge with DNFB or urushiol (i.e., day 6 after sensitization) mast-cell-deficient $Kit^{W}/Kit^{W\text{-}v}$ or $Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$ mice exhibit ear swelling responses similar to those of the corresponding WT littermates; these responses are associated with little evidence of mast-cell activation in WT mice[9]. (**H**) Five d after epicutaneous challenge with DNFB or urushiol, when the mice have elevated circulating levels of Ag-specific IgG1 antibodies, mast-cell-derived IL-10 contributes to the ability of mast cells to limit numbers of innate inflammatory cells and T cells, and tissue pathology, at the site of hapten challenge[9]. Based on *in vitro* studies, we speculate that increased local expression of certain cytokines (e.g., IL-4) at the site of hapten challenge

can increase mast-cell surface expression of FcγRIII, and perhaps have other effects on mast-cell phenotype/function; such mast cells then exhibit enhanced ability to secrete TNF and IL-10 upon stimulation, *via* their FcγRIII, with immune complexes of specific Ag and IgG1 antibodies[9]. (**G**) In the absence of mast cells (and mast-cell-derived IL-10), the pathology associated with these CHS responses is substantially exacerbated and there is increased inflammation, marked thickening of the epidermis, increases in extracellular matrix (ECM) and other components of the dermis, as well as areas of full thickness epidermal necrosis and ulceration[9].

Supplemental Figure 1. Potential functions of mast-cell TNF



Supplemental Figure 1. Potential functions of mast-cell TNF. Some of the potential functions of TNF are illustrated. Most of the evidence cited is derived from studies of TNF *in vitro*. Because mast cells can release TNF from pre-formed stores rapidly upon appropriate activation, in addition to later producing TNF from new transcripts[11-15], mast cells have the potential to supply TNF both at early and later intervals of many innate or acquired immune responses. TNF has the potential to influence structural cells via direct effector functions, e.g., by enhancing expression of adhesion molecules (ICAM-1, E-selectin and VCAM-1) on vascular endothelial cells[15,16], altering fibroblast collagen production[17-19], promoting fibroblast proliferation[20] or monocyte chemoattractant protein-1 (MCP-

1) production[21,22] or activating granulocytes and monocytes/macrophages[23]. TNF also has the potential to influence structural cells via immunomodulatory functions. For example, the ability of TNF to increase adhesion molecule expression on vascular endothelial cells can promote leukocyte recruitment; recruited leukocytes can then exert effector functions at that site, such as increasing fibroblast proliferation and collagen production[24,25]. *In vitro* studies, as well as a few *in vivo* studies, suggest that mast-cell-derived TNF can mediate direct or indirect immunomodulatory functions on immune cells. For example, the soluble form of mast-cell-derived TNF can promote optimal T cell activation by mast cells in some settings[26], while in certain acquired immune responses mast-cell membrane-associated TNF can promote optimal dendritic cell (DC) migration to draining lymph nodes[27]. Mast-cell-derived TNF can have indirect immunomodulatory functions as well, such as when DCs stimulated with mast-cell-derived TNF then interact with T cells that in turn regulate the immune response. As indicated in the figure by “?” symbols, some effects of mast-cell-derived TNF, such as those that promote elongation of dermal and epidermal nerves, may reflect a complex combination of effector and immunomodulatory actions of this cytokine[28].

References

1. Khan, A.I., Horii, Y., Tiuria, R., Sato, Y. & Nawa, Y. Mucosal mast cells and the expulsive mechanisms of mice against Strongyloides venezuelensis. *Int J Parasitol* **23**, 551-5 (1993).
2. Lantz, C.S. et al. Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* **392**, 90-3 (1998).
3. Cassatella, M.A. The neutrophil: one of the cellular targets of interleukin-10. *Int J Clin Lab Res* **28**, 148-61 (1998).
4. Erwig, L.P., Kluth, D.C., Walsh, G.M. & Rees, A.J. Initial cytokine exposure determines function of macrophages and renders them unresponsive to other cytokines. *J Immunol* **161**, 1983-8 (1998).
5. Becherel, P.A. et al. Interleukin-10 inhibits IgE-mediated nitric oxide synthase induction and cytokine synthesis in normal human keratinocytes. *Eur J Immunol* **25**, 2992-5 (1995).
6. Beissert, S., Schwarz, A. & Schwarz, T. Regulatory T cells. *J Invest Dermatol* **126**, 15-24 (2006).
7. O'Garra, A. & Vieira, P. T(H)1 cells control themselves by producing interleukin-10. *Nat Rev Immunol* **7**, 425-8 (2007).
8. Muller, G. et al. Interleukin-10-treated dendritic cells modulate immune responses of naive and sensitized T cells in vivo. *J Invest Dermatol* **119**, 836-41 (2002).
9. Grimbaldeston, M.A., Nakae, S., Kalesnikoff, J., Tsai, M. & Galli, S.J. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat Immunol* **8**, 1095-104 (2007).
10. Bryce, P.J. et al. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity* **20**, 381-92 (2004).
11. Young, J.D., Liu, C.C., Butler, G., Cohn, Z.A. & Galli, S.J. Identification, purification, and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. *Proc Natl Acad Sci U S A* **84**, 9175-9 (1987).
12. Klein, L.M., Lavker, R.M., Matis, W.L. & Murphy, G.F. Degranulation of human mast cells induces an endothelial antigen central to leukocyte adhesion. *Proc Natl Acad Sci U S A* **86**, 8972-6 (1989).
13. Gordon, J.R. & Galli, S.J. Mast cells as a source of both preformed and immunologically inducible TNF-alpha/cachectin. *Nature* **346**, 274-6 (1990).
14. Gordon, J.R. & Galli, S.J. Release of both preformed and newly synthesized tumor necrosis factor alpha (TNF-alpha)/cachectin by mouse mast cells stimulated via the Fc epsilon RI. A mechanism for the sustained action of mast cell-derived TNF-alpha during IgE-dependent biological responses. *J Exp Med* **174**, 103-7 (1991).
15. Walsh, L.J., Trinchieri, G., Waldorf, H.A., Whitaker, D. & Murphy, G.F. Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci U S A* **88**, 4220-4 (1991).

16. Clark, P.R., Pober, J.S. & Kluger, M.S. Knockdown of TNFR1 by the sense strand of an ICAM-1 siRNA: dissection of an off-target effect. *Nucleic Acids Res* **36**, 1081-97 (2008).
17. Chou, D.H., Lee, W. & McCulloch, C.A. TNF-alpha inactivation of collagen receptors: implications for fibroblast function and fibrosis. *J Immunol* **156**, 4354-62 (1996).
18. Mauviel, A. et al. Tumor necrosis factor inhibits collagen and fibronectin synthesis in human dermal fibroblasts. *FEBS Lett* **236**, 47-52 (1988).
19. Solis-Herruzo, J.A., Brenner, D.A. & Chojkier, M. Tumor necrosis factor alpha inhibits collagen gene transcription and collagen synthesis in cultured human fibroblasts. *J Biol Chem* **263**, 5841-5 (1988).
20. Kendall, J.C., Li, X.H., Galli, S.J. & Gordon, J.R. Promotion of mouse fibroblast proliferation by IgE-dependent activation of mouse mast cells: role for mast cell tumor necrosis factor-alpha and transforming growth factor-beta 1. *J Allergy Clin Immunol* **99**, 113-23 (1997).
21. Gordon, J.R. Monocyte chemoattractant peptide-1 expression during cutaneous allergic reactions in mice is mast cell dependent and largely mediates the monocyte recruitment response. *J Allergy Clin Immunol* **106**, 110-6 (2000).
22. Gordon, J.R. TGFbeta1 and TNFalpha secreted by mast cells stimulated via the Fc epsilon RI activate fibroblasts for high-level production of monocyte chemoattractant protein-1 (MCP-1). *Cell Immunol* **201**, 42-9 (2000).
23. Larrick, J.W. et al. Recombinant tumor necrosis factor causes activation of human granulocytes. *Blood* **69**, 640-4 (1987).
24. Postlethwaite, A.E., Smith, G.N., Mainardi, C.L., Seyer, J.M. & Kang, A.H. Lymphocyte modulation of fibroblast function in vitro: stimulation and inhibition of collagen production by different effector molecules. *J Immunol* **132**, 2470-7 (1984).
25. Lee, H.G., Choi, I., Pyun, K.H. & Park, K.W. Peritoneal lavage fluids stimulate NIH3T3 fibroblast proliferation and contain increased tumour necrosis factor and IL-6 in experimental silica-induced rat peritonitis. *Clin Exp Immunol* **100**, 139-44 (1995).
26. Nakae, S. et al. Mast cells enhance T cell activation: importance of mast cell costimulatory molecules and secreted TNF. *J Immunol* **176**, 2238-48 (2006).
27. Suto, H. et al. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* **176**, 4102-12 (2006).
28. Kakurai, M. et al. Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice. *Am J Pathol* **169**, 1713-21 (2006).