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CD40–CD40L interactions in atherosclerosis

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The abundant presence of macrophages and activated T cells in atherosclerotic plaques suggests an active involvement of the immune system in this cardiovascular disease. However, insight into cellular interactions inducing relevant effector functions is still limited. Here, Jon Laman and colleagues propose that local interactions between CD40 and CD40 ligand may induce multiple activities by different cell types contributing to atherosclerosis.

Atherosclerosis is the result of an intricate interplay between diverse factors such as lipid metabolism, blood coagulation elements, cytokines, hemodynamic stress and behavioral risk factors. There are two main hypotheses that could explain the initiation of atherosclerosis: modification of low-density lipoprotein (LDL) and an inappropriate immune response to vascular injury. LDL modified by oxidation (oxLDL) in macrophage-rich tissues is present in large amounts in atherosclerotic plaques, where it stimulates T-cell migration, is immunogenic and induces antibody production (reviewed in Ref. 1). It has long been recognized that large numbers of activated $CD4^+$ T cells and macrophages are present within inflammatory atherosclerotic plaques, and recent findings have strengthened the case for an important role of (auto)immunity[1–4]. However, this concept is still a matter for debate. For instance, the role of the immune system has been questioned following the finding that mice either with severe combined immunodeficiency, with deficiencies in major histocompatibility (MHC) class I or class II expression, or lacking a thymus, nevertheless develop characteristic atherosclerotic lesions with monocyte infiltration[5].

The central issues relating to possible autoimmunity in atherosclerosis concern the nature of the antigens involved and the cellular interactions that are required to initiate relevant effector functions. Atherosclerosis-inducing antigens have yet to be unequivocally identified, but oxLDL, viral antigens and heat shock proteins have been suggested to elicit immune responses, some of which might fulfill protective roles instead of pathogenic ones[1,6]. Although the involvement of adhesion molecules in cellular interactions has been studied in detail, insight into local communication between the major players within atherosclerotic plaques (endothelium, T cells, macrophages and smooth muscle cells) is still minimal.

The CD40–CD40L receptor–ligand pair plays a central role in antigen presentation and autoimmunity, as well as in T-cell and macrophage activation[7,8], and may also mediate cellular interactions involved in atherosclerosis. CD40–CD40L interactions mediate inflammatory responses that could serve to accelerate the atherosclerotic process. This article examines direct and indirect evidence for this hypothesis, and discusses its experimental and therapeutic implications.

CD40 is expressed in human atherosclerotic lesions

Immunohistochemical analysis of CD40 in human tissues has provided experimental support for the hypothesis that CD40–CD40L interactions are operational in atherosclerosis. Staining for the presence of CD40 in endarterectomy specimens from carotid arteries of patients treated surgically for occlusive carotid disease shows that CD40 is expressed by separate cell morphologies within lesions (Fig. 1a,b). As the number of B cells present is usually very low, macrophages seem the prime candidate cell type to express CD40 within atherosclerotic lesions. This is confirmed by double staining for CD40 and acid phosphatase, a lysosomal enzyme characteristic for differentiated macrophages. Figure 1c shows that cells positive for CD40 are singly present, and can be found in the vicinity of cells with acid phosphatase activity. These $CD40^+$ cells may represent monocytes or immature macrophages, or another cell type, such as B cells or (myo)fibroblasts, which can also express CD40 (see below). However, as can be seen in Fig. 1d, at least a fraction of inflammatory cells co-expresses CD40 on the membrane and acid phosphatase in the cytoplasm, and these cells therefore belong to the monocyte/macrophage lineage. In summary, CD40 is present in atherosclerotic lesions, and is expressed by macrophages and possibly also by other cell types.

The abundant expression of CD40 in atherosclerotic lesions supports the hypothesis that this molecule acts in cardiovascular disease, and calls for an evaluation of possible functional effects of CD40 ligation within diseased arteries. Pertinent immunological aspects of atherosclerotic lesions[6,9,10] are summarized below, followed by a functional interpretation of putative CD40–CD40L interactions in atherosclerosis (see also Box 1).

Immunology of atherosclerotic plaques

In early atherogenesis, monocytes adhere to intact endothelium and gain access to the intima[9]. In later stages, monocytes may preferentially adhere to sites of endothelial injury. Macrophages accumulate cholesterol and often transform into foam cells. In advanced atherosclerosis, a necrotic core is formed containing cell debris and lipids, probably resulting from death of macrophages and foam cells. Endogenous and infiltrating macrophages proliferate locally, and functional subsets may develop. Smooth muscle cells also proliferate within lesions. Macrophages are abundant in the lateral

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Fig. 1. *CD40 expression in human atherosclerosis. (a) CD40-expressing cells (red; arrowheads) in an atherosclerotic lesion (magnification = ×80). Control stainings omitting the primary antibody or using an isotype-matched control antibody of irrelevant specificity were negative. (b) CD40-expressing cells (red) in an atherosclerotic lesion (magnification = ×80); note different cellular morphology compared with (a). (c) CD40-expressing cells (blue; arrowheads) in an atherosclerotic lesion, localized in the vicinity of macrophages with acid phosphatase activity (red) (magnification = ×200). (d) CD40 (blue) is expressed by macrophages having intracytoplasmic acid phosphatase activity (red), resulting in double staining (magnification = ×500); these macrophages have morphological characteristics of foam cells. All photomicrographs represent frozen sections of carotis material obtained from atherosclerosis patients. Immunohistochemical staining for CD40 and histochemical staining for acid phosphatase were performed as described in Ref. 21.*

margins of the fibrous cap of the lesion, where ruptures are most likely to occur, leading to thrombosis, occlusion of the artery and hemorrhage. A significant percentage of cells within lesions are proliferating polyclonal T cells, of which approximately two-thirds are $CD4^+$ and the rest are $CD8^+$. The $CD4^+$ T cells are memory cells, characterized by expression of CD45R0, MHC class II, very late activation antigen 1 (VLA-1) and interleukin 2 receptor α (IL-2Rα; CD25)[6]. Although this phenotype is compatible with CD40L expression, this has not been addressed in detail in atherosclerosis. No natural killer (NK) cells are found, and macrophages outnumber T cells by 10–50:1. Mast cells, which can express CD40L, are also found.

There is little evidence for cytotoxic reactions within plaques. Proinflammatory cytokines such as IL-1, IL-6, tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) are secreted in the plaque, presumably by one or more of the following cell types: T cells, macrophages, endothelial cells and smooth muscle cells. Macrophages also produce platelet-derived growth factor (PDGF), monocyte chemotactic protein 1 (MCP-1), macrophage colony-stimulating factor (M-CSF), matrix metalloproteinases (MMPs) such as collagenase and gelatinase B, and nitric oxide (NO)[10]. Apart from cytokines, T cells within plaques produce heparin-binding epidermal-growth-factor-like factor (HB-EGF) and basic fibroblast growth factor (bFGF), contributing to smooth muscle cell hyperplasia[11]. Both $CD8^+$ and $CD4^+$ T helper 1 (Th1) cells, but not Th2 cells, can help production of tissue factor and procoagulant activity by macrophages[12]. IL-12 is induced in monocytes in response to oxLDL, and IL-10 inhibits this release[13], indicating crossregulatory roles for these cytokines.

It is clear that the roles of macrophages and T cells in atherosclerosis are intimately intertwined and complex. Furthermore, cellular interactions and secretion of soluble compounds are dependent on the combination of conditions in the microenvironment[6,9,10].

Putative roles of CD40–CD40L in atherosclerosis

CD40L on activated T cells

There is evidence indicating that ligation of CD40L on activated T cells is required for T-cell priming[7]. In addition, CD40L ligation strongly enhances production of Th1 cytokines (IL-2, IFN-γ) and Th2 cytokines (IL-4, IL-5 and IL-10)[14] (see also Table 1). These findings demonstrate that CD40–CD40L interactions mediate the

Box 1. Putative roles of the CD40–CD40L interaction in atherosclerosis[a]

T cell–endothelium
T-cell adhesion to endothelium of inflamed tissue
Increased expression of E-selectin, VCAM-1 and ICAM-1 on endothelium
Increased expression of CD40L by T cells
Production of metalloproteinases by T cells, facilitating migration

T cell–macrophage
Increased expression of ICAM-1, MHC class II, B7-2 and CD40 on macrophages
Improved antigen presentation by macrophages
Production of proinflammatory cytokines by macrophages
Production of nitric oxide by macrophages
Production of metalloproteinases by macrophages
T-cell priming[b]
Production of cytokines by T cells[b]

T cell–B cell
B-cell proliferation
Isotype switching
Antibody production (cytolysis, ADCC, opsonization)

T cell–smooth muscle cell
Antigen presentation

T cell–(myo)fibroblast
Proliferation of (myo)fibroblasts
Tissue restructuring
Increased expression of ICAM-1 and VCAM-1 by (myo)fibroblasts
Production of IL-6 by (myo)fibroblasts

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CD40L, CD40 ligand; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin 6; MHC, major histocompatibility complex; VCAM-1, vascular cell adhesion molecule 1.
[a]Activated $CD4^+$ T cells express CD40L and can interact with CD40 expressed on the cells described above. Mast cells can also express CD40L, can be present in atherosclerotic lesions, and may interact with one or more of the CD40-expressing cells described above.
[b]T-cell priming and ensuing cytokine production may also result from the interaction of T cells with cell types other than macrophages expressing CD40.

signaling that eventually affects the activation state of both the antigen-presenting cell (APC) and the T cell. In atherosclerotic plaques, triggering of CD40L on T cells may therefore assist in regulation of T-cell expansion and cytokine production. It is of note that T cells that migrate to the plaque intima may have undergone primary sensitization in the draining lymph nodes; for these T cells, restimulation would take place in the lesion.

CD40 on endothelium

CD40 has been detected on different types of inflamed and normal endothelium[15,16]. Ligation of CD40 induces expression of E-selectin (CD62E), vascular cell adhesion molecule 1 (VCAM-1; CD106) and intercellular adhesion molecule 1 (ICAM-1; CD54), but not B7-1 (CD80), B7-2 (CD86) or MHC class II. No production of IL-6 or granulocyte–macrophage colony-stimulating factor (GM-CSF) was found in response to CD40 ligation. CD40 on endothelium can augment the expression of CD40L by a pathway dependent on CD2 interaction with leukocyte function-associated molecule 3 (LFA-3). Therefore, in the case of atherosclerosis, interaction of $CD40L^+$ T cells with $CD40^+$ endothelial cells may upregulate expression of CD40L on the T cells and adhesion molecules on the endothelium, improving migration of mononuclear effector cells into the plaque. In this respect, it will be of interest to determine whether CD40-mediated triggering of $CD40L^+$ T cells is involved in the production of metalloproteinases MMP-2 and MMP-9, which facilitate both the extravasation of lymphocytes, by degradation of the basement membrane, and their migration through connective tissues[17].

CD40 on B cells

As mentioned above, the presence of B cells within atherosclerotic lesions is generally rare, making it unlikely that locally produced antibody plays an important role. However, there are some exceptions: for instance, in periaortitis (inflammation of the outer coat of the aorta), B cells dominate in periadventitial inflammation[6]. In such cases, CD40L signaling of B cells may contribute to local (auto)antibody production and possibly extrafollicular isotype switching. Furthermore, heat shock protein 65 (Hsp65) and oxLDL are potential targets for antibody-mediated cytolytic responses[1,18].

CD40 on fibroblasts

Information on the role of CD40 expressed by fibroblasts is still limited, but does indicate that ligation functionally activates fibroblasts. IFN-γ (but not IL-4) secreted by activated T cells induces upregulation of CD40 expression, and ligation of CD40 leads to mobilization of NF-κB, proliferation of fibroblasts, upregulation of the adhesion molecules CD54 and CD106, and production of IL-6. Expression of CD40 is higher on nonproliferating fibroblasts[19,20], indicating that CD40 could act as a co-regulator of fibroblast proliferation. This suggests that CD40 on fibroblasts is linked both to tissue repair, including collagen production, and antigen presentation to T cells. Therefore, CD40 ligation in atherosclerosis may contribute to the antigen-presenting function and the tissue-restructuring capacity of local (myo)fibroblasts.

CD40 on macrophages

Evidence from *in vivo* studies indicates that CD40 on macrophages is involved in chronic autoimmune disease: CD40 is expressed at high levels by macrophages infiltrating the brain in multiple sclerosis[21] and during experimental autoimmune encephalomyelitis

(EAE) in mice and marmoset monkeys (J.D. Laman *et al.*, unpublished). A wide range of *in vitro* studies has shown that ligation of CD40 expressed by macrophages can activate a series of effector functions relevant to chronic inflammation of arteries.

CD40 ligation on macrophages elicits adherence to CD40L-expressing cells, homotypic aggregation, increased survival of cells in culture and tumoricidal activity[22]. In addition, antigen-presenting capacities of macrophages are improved by increased expression of CD54, MHC class II, CD86 and CD40 itself[23]. CD40L supplies a co-stimulatory signal for macrophages to produce the proinflammatory cytokines TNF-α, IL-1β, IL-6, IL-8 and the p40 moiety of IL-12 (Refs 24–26). Finally, CD40 ligation can enhance NO production by macrophages[27].

Interestingly, a recent study has demonstrated that CD40 ligation can induce production of the MMP gelatinase by macrophages. This effect was significantly enhanced by IFN-γ from activated $CD4^+$ T cells[28]. In addition, a cell-surface molecule (probably CD40L) on activated T cells induces production of interstitial collagenase in monocytes and fibroblasts[29]. In general, macrophages are able to secrete the full spectrum of MMPs to mediate degradation, removal and remodeling of connective tissues. Macrophages, which are present in high numbers in the shoulders of atherosclerotic plaques, have been found to break down collagen in the fibrous cap by means of MMP-1 and MMP-2 (Ref. 30); this might contribute to the rupturing of the lesion, which usually occurs at this site. In addition, MMPs are involved in release of transforming growth factor β1 (TGF-β1) and insulin-like growth factor (IGF) from extracellular storage sites, and in cleavage of TNF and Fas ligand (FasL) from the surface of activated macrophages and T cells. By stimulating MMP production, CD40 signaling is therefore potentially involved in remodeling of the plaque and regulation of mononuclear cell activity.

In summary, there is strong evidence that ligation of CD40 on macrophages can lead to improved antigen presentation, as well as secretion of MMPs, proinflammatory cytokines and NO. All of these phenomena are directly relevant to the atherosclerotic process.

Table 1. CD40–CD40L-stimulated production of compounds involved in atherosclerosis[a,b]

Compound	T cells	Endothelium	Macrophages	Fibroblasts
IL-1	–	–	+	–
IL-2	+	–	–	–
IL-4	+	–	–	–
IL-5	+	–	–	–
IL-6	–	–	+	+
IL-8	–	–	+	–
IL-10	+	–	–	–
IL-12	–	–	+	–
TNF-α	–	–	+	–
IFN-γ	–	–	–	–
MMP-1 (collagenase)	+	–	–	–
MMP-9 (gelatinase B)	–	–	+	–
NO	–	–	+	–

Abbreviations: CD40L, CD40 ligand; IFN-γ, interferon γ; IL, interleukin; MMP, matrix metalloproteinase; NO, nitric oxide; TNF-α, tumor necrosis factor α.
[a]+ indicates CD40–CD40L-stimulated production observed in *in vitro* and *in vivo* studies not directly related to atherosclerosis; – indicates no production or unknown.
[b]Smooth muscle cells have not been included in this table as CD40 expression by these cells has not yet been demonstrated.

Testing the hypothesis

The evidence discussed above supports a role for CD40–CD40L in atherosclerosis. Further substantiation of this role requires detailed evaluation of local expression of these molecules in human and animal atherosclerotic material. Whereas local expression of CD40L would be expected on T cells and possibly mast cells, CD40 is probably present on endothelium, macrophages and (myo)fibroblasts. Whether some smooth muscle cells might express CD40 under appropriate conditions at specific sites is unknown at present, but they are able to express MHC class II (Ref. 6), which correlates with CD40 expression in most APC types.

In order to correlate expression and function, further *in situ* analysis should take into account several sources of variation; for instance, lesions may present differently depending on the type of atherosclerotic disease. In addition, the stage of the disease, the age of the lesion and the different compartments of the lesion (for example necrotic core versus fibrous cap) will affect CD40 expression. The same is true for the maturation state of functional macrophage subsets that may operate locally. Ideally, the earliest stages of the disease, even before the occurrence of fatty streaks, should be included in this analysis.

An important issue is the possible antigen dependence of CD40–CD40L interactions. In general, antigenic stimulation is required for T cells to express CD40L; however, it can be envisaged that an appropriate (inflamed) microenvironment can induce CD40L in the absence of antigenic stimulation, and that CD40L$^+$ T cells activate CD40$^+$ cells independently of simultaneous antigen presentation. Indeed, a recent study[31] described a mechanism that maintains an antigen-nonspecific inflammatory response involving CD40: soluble CD23 directly activates monocytes to stimulate resting T cells in an antigen-independent fashion by means of CD40–CD40L interaction. This signal costimulates IL-2- or IL-12-induced IFN-γ production by the T cells, perpetuating inflammation, and involving TNF-α.

The *in situ* evaluation of CD40 and CD40L expression should be complemented by identification *in vitro* of the full spectrum of cytokines, growth factors and chemoattractants produced by the major cell types following CD40 ligation *in vitro*. It will be of major interest to determine whether CD40 ligation promotes secretion of molecules such as PDGF and vascular endothelial growth factor (VEGF). In addition, the possibility of CD40 expression by smooth muscle cells and its implications should be investigated. As an *in vivo* model, gene-targeted mice deficient in CD40 or CD40L could be backcrossed with existing mouse models for atherosclerosis, such as mice deficient in or overexpressing apolipoprotein E. Novel strategies that are currently being developed for inducible gene targeting and tissue-specific expression of CD40 (for instance restricted to macrophages by means of the lysozyme promotor) will provide further insight into the roles of this molecule in vascular disease.

It should be emphasized that not all effects of CD40–CD40L interactions need necessarily be pathogenic in atherosclerosis. Tissue repair and restructuring, facilitation of cell migration, and stimulation of the scavenger function of macrophages could be among the beneficial actions of this receptor pair.

Therapeutic implications

If a major contribution of the CD40–CD40L interaction to chronic inflammatory cardiovascular disease is confirmed and its mechanisms elucidated, therapeutic strategies designed to modulate this interaction will provide a useful addition to current treatment regimens; such strategies might include fusion proteins or small molecule inhibitors. Optimally, therapy should be initiated in early stages of lesion formation. However, these approaches suffer from common drawbacks: identifying patients at risk, early diagnosis, bioavailability of the drug, access to the plaque and induction of undesired antibody responses. In addition, because CD40 is expressed ubiquitously and most notably in the lymphoid system, side-effects may ensue from CD40–CD40L-targeted therapy, such as reduced antibody responses against thymus-dependent antigens.

A more unorthodox approach to therapy is to manipulate the CD40–CD40L interaction to induce peripheral T-cell tolerance. Providing the autoantigenic signal in the absence of costimulation by blocking this receptor–ligand interaction can silence T cells with autoreactive potential. Although this approach has been successful in other experimental systems (reviewed in Ref. 8), it requires the unequivocal identification of autoantigens in atherosclerosis.

Note added in proof: CD40L can be functionally expressed on human vascular endothelial cells, smooth muscle cells and macrophages in atherosclerosis (Mach, F. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 1931–1936), lending further support to the hypothesis described in this article.

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References

1 Wick, G., Schett, G., Amberger, A., Kleindienst, R. and Xu, Q. (1995) *Immunol. Today* 16, 27–33

2 Zhou, X., Stemme, S. and Hansson, G.K. (1996) *Am. J. Pathol.* 149, 359–366

3 Emeson, E.E., Shen, M-L., Bell, C.G.H. and Qureshi, A. (1996) *Am. J. Pathol.* 149, 675–685

4 Lichtman, A.H., Cybulsky, M. and Luscinskas, F.W. (1996) *Am. J. Pathol.* 149, 351–357

5 Fyfe, A.I., Qiao, J-H. and Lusis, A.J. (1994) *J. Clin. Invest.* 94, 2516–2520

6 Hansson, G.K. and Libby, P. (1996) in *Atherosclerosis and Coronary Artery Disease* (Vol. 1) (Fuster, V., Ross, R. and Topol, E.J., eds), pp. 557–568, Lippincott-Raven Publishers

7 Grewal, I.S. and Flavell, R.A. (1996) *Immunol. Today* 17, 410–414

8 Laman, J.D., Claassen, E. and Noelle, R.J. (1996) *Crit. Rev. Immunol.* 16, 59–108

9 Raines, E.W., Rosenfeld, M.E. and Ross, R. (1996) in *Atherosclerosis and Coronary Artery Disease* (Vol. 1) (Fuster, V., Ross, R. and Topol, E.J., eds), pp. 539–555, Lippincott-Raven Publishers

10 Libby, P. and Ross, R. (1996) in *Atherosclerosis and Coronary Artery Disease* (Vol. 1) (Fuster, V., Ross, R. and Topol, E.J., eds), pp. 585–594, Lippincott-Raven Publishers

11 Peoples, G.E., Blotnick, S., Takahashi, K., Freeman, M.R., Klagsbrun, M. and Eberlein, T.J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 6547–6551

12 Del Prete, G., De Carli, M., Lammel, R.M. *et al.* (1995) *Blood* 86, 250–257

13 Uyemura, K., Demer, L.L., Castle, S.C. *et al.* (1996) *J. Clin. Invest.* 97, 2130–2138

14 Peng, X., Kasran, A., Warmerdam, P.A.M., de Boer, M. and Ceuppens, J.L. (1996) *Eur. J. Immunol.* 26, 1621–1627

15 Karmann, K., Hughes, C.C., Schechner, J., Fanslow, W.C. and Pober, J.S. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 4342–4346

16 Hollenbaugh, D., Mischel-Petty, N., Edwards, C.P. *et al.* (1995) *J. Exp. Med.* 182, 33–40

17 Goetzl, E.J., Banda, M.J. and Leppert, D. (1996) *J. Immunol.* 156, 1–4

18 Schett, G., Xu, Q., Amberger, A. *et al.* (1995) *J. Clin. Invest.* 96, 2569–2577

19 Yellin, M.J., Winikoff, S., Fortune, S.M. *et al.* (1995) *J. Leukocyte Biol.* 58, 209–216

20 Fries, K.M., Sempowski, G.D., Gaspari, A.A., Blieden, T., Looney, R.J. and Phipps, R.P. (1995) *Clin. Immunol. Immunopathol.* 77, 42–51

21 Gerritse, K., Laman, J.D., Noelle, R.J. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 2499–2504

22 Alderson, M.R., Armitage, R.J., Tough, T.W., Strockb e, L., Fanslow, W.C. and Spriggs, M.K. (1993) *J. Exp. Med.* 178, 669–674
23 Kiener, P.A., Moran-Davis, P., Rankin, B.M., Wahl, A.F., Aruffo, A. and Hollenbaugh, D. (1995) *J. Immunol.* 155, 4917–4925
24 Kennedy, M.K., Picha, K.S., Fanslow, W.C. *et al.* (1996) *Eur. J. Immunol.* 26, 370–378
25 Wagner, D.H., Stout, R.D. and Suttles, J. (1994) *Eur. J. Immunol.* 24, 3148–3154
26 Kato, T., Hakamada, R., Yamane, H. and Nariuchi, H. (1996) *J. Immunol.* 156, 3932–3938
27 Tian, L., Noelle, R.J. and Lawrence, D.A. (1995) *Eur. J. Immunol.* 25, 306–309
28 Malik, N., Greenfield, B.W., Wahl, A.F. and Kiener, P.A. (1996) *J. Immunol.* 156, 3952–3960
29 Miltenburg, A.M.M., Lacraz, S., Welgus, H.G. and Dayer, J-M. (1995) *J. Immunol.* 154, 2655–2667
30 Dollery, C.M., McEwan, J.R. and Henney, A.M. (1995) *Circ. Res.* 77, 863–868
31 Armant, M., Rubio, M., Delespesse, G. and Sarfati, M. (1995) *J. Immunol.* 155, 4868–4875

IL-10: a potential therapy for allergic inflammation?

Marina Pretolani and Michel Goldman

Interleukin 10 (IL-10) is currently regarded as a potential therapy for inflammatory diseases involving T helper 1 (Th1)-type responses because of its ability to downregulate several major functions of Th1 cells and macrophages. Here, evidence is provided that IL-10 could also be useful in controlling Th2-mediated inflammatory processes by preventing the accumulation of activated eosinophils in target tissues.

Interleukin 10 (IL-10) was originally characterized as a factor generated by mouse T helper 2 (Th2) cells that inhibits cytokine synthesis by Th1 cells[1]. Subsequently, IL-10 was shown to downregulate the synthesis of a broad spectrum of proinflammatory cytokines by monocytes/macrophages[2] and neutrophils[3], and to promote the release of the IL-1 receptor antagonist (IL-1ra) by these cells[4]. Such *in vitro* data led to the proposal that IL-10 might inhibit inflammatory processes mediated by Th1 cells *in vivo*. Indeed, systemic administration of IL-10 in rodents suppresses delayed-type hypersensitivity[5,6], experimental autoimmune encephalomyelitis[7] and T-cell-mediated inflammatory bowel disease[8]. This article discusses recent findings indicating that IL-10 might also be used to prevent allergic inflammation induced by Th2 cells. This might occur by inhibition of the production of cytokines involved in the differentiation, activation and recruitment of eosinophils and by direct suppressive effects on eosinophils and mast cells.

Regulation of allergic eosinophilic inflammation

Tissue inflammation related to the accumulation of eosinophils is a characteristic feature of allergic diseases, such as bronchial asthma and atopic dermatitis. In asthma, activated $CD4^+$ T cells, eosinophils and mast cells are found in the bronchial tissue and bronchoalveolar lavage fluid (BALF), and their numbers correlate with the severity of the disease[9,10]. Further studies identifying an increased number of T cells expressing mRNA for IL-4 and IL-5, but not interferon γ (IFN-γ), in the BALF from asthmatic patients[11] has lent support to the hypothesis that a Th2-mediated inflammatory response is associated with this disease. The infiltration and maintenance of eosinophils in the inflammatory foci is governed mostly by T-cell-derived cytokines. These include IL-3, IL-5 and granulocyte–macrophage colony-stimulating factor (GM-CSF), which act on the proliferation and differentiation of eosinophils, promote chemotaxis of mature cells and prime them for their responses to exogenous stimuli[12,13].

In the past few years, it has become apparent that activated mast cells produce a similar pattern of cytokines to that of Th2 cells[14–17], leading to the concept that these cells exert many effects relevant to allergic inflammation. Mast cells also generate tumor necrosis factor α (TNF-α), which interferes directly with leukocyte chemoattraction, activation and survival, but also indirectly, by inducing the expression of specific adhesion molecules[18] that facilitate the access of leukocytes to inflamed tissues. Although T cells and mast cells may represent the initial source of eosinophilotactic cytokines during allergic reactions, production of IL-4, IL-5, GM-CSF and TNF-α by the eosinophils themselves also participates in the maintenance of local eosinophilia[19].

The identification of a family of eosinophilotactic chemokines has provided a potential new mechanism to explain the selective recruitment of eosinophils into target tissues during allergic inflammation[20]. Chemokines RANTES and monocyte chemotactic protein 3

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