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A role for secretory phospholipase A₂ and C-reactive protein in the removal of injured cells

C. Erik Hack, Gert-Jan Wolbink, Caspar Schalkwijk, Han Speijer, Willem Th. Hermens and Henk van den Bosch

The acute phase response is initiated in response to infection or physical trauma and is characterized by an increase in the levels of some plasma proteins. Here, Erik Hack and colleagues suggest that the combined actions of two of these acute phase proteins, secretory phospholipase A₂ and C-reactive protein, may serve to promote phagocytosis of injured cells and tissue debris, thereby enhancing inflammation and tissue damage.

The acute phase response (APR) is a well-known clinical phenomenon characterized by leukocytosis, fever, alterations in the metabolism of many organs, and changes in the plasma concentrations of various so-called acute phase proteins (APPs)¹⁻³. Although the APR is preserved throughout vertebrate evolution and can be induced by numerous immunological and nonimmunological events, its function is not well understood. Many investigators believe that the main function of the APR is to prevent ongoing tissue damage, to neutralize the inflammatory agent and to activate repair processes². The prototypal APP in humans is C-reactive protein (CRP); plasma levels of this APP start to rise as early as about 8–10 h after challenge with the inciting stimulus⁴. In addition, circulating levels of the enzyme secretory phospholipase A₂ (sPLA₂) start to rise approximately 6–8 h after challenge⁵. sPLA₂ has generally been considered to play a role in the pathway leading to the formation of eicosanoids⁶. However, as discussed below, this may not be the main activity of the enzyme; rather, the function of sPLA₂ may be related to that of CRP such that their combined effects serve to promote phagocytosis of injured cells and tissue debris by neutrophils.

sPLA₂

PLA₂ hydrolyzes the sn-2-ester bond of phospholipids, generating arachidonic acid⁶, and thus catalyzes the rate-limiting step in the formation of eicosanoids⁷. In humans, different forms of PLA₂ have been identified: several cytosolic forms^{8,9}, and two secreted forms, type I PLA₂ secreted by the pancreas, and type II (further denoted as sPLA₂) produced by many cells including liver cells¹⁰.

The hepatocarcinoma cell line HepG2 represents a suitable *in vitro* model to study the regulation of APP synthesis. In these cells, sPLA₂ synthesis is greatly stimulated by cytokines such as interleukin 1β (IL-1β), IL-6 and tumor necrosis factor α (TNF-α)¹⁰. IL-6 and other cytokines are known to be released by macrophages, endothelial cells and fibroblasts at sites of tissue injury. The regulatory sequences of the gene encoding sPLA₂ contain a putative IL-6-

responsive element, homologous to that found in several other genes encoding APPs (Ref. 10). In addition, the intravenous administration of IL-1β, TNF-α or IL-6 induces a 10–100-fold increase of circulating sPLA₂, starting within 3–4 h and reaching peak levels after approximately 10 h (G.-J. Wolbink *et al.*, unpublished). These data, together with the finding that circulating sPLA₂ is increased in many inflammatory diseases^{5,11}, suggest that sPLA₂ should be considered as an APP. Indeed, the role of sPLA₂ in the generation of eicosanoids may not be its principal function. It is particularly unclear why

cells should secrete an enzyme that is involved in an intracellular metabolic process when they can simply synthesize other cytosolic PLA₂ enzymes⁷.

Flip-flopped membranes

Phospholipids are asymmetrically distributed among the inner and outer leaflets of the intact cell membrane: the outer leaflet primarily contains sphingomyelin and phosphatidylcholine (PC), whereas the inner leaflet harbors most of the phosphatidylserine (PS) and phosphatidylethanolamine (PE) (Fig. 1)¹². Maintenance of this asymmetry is energy dependent and requires adenosine triphosphate (ATP). In the absence of ATP, the phospholipids of the inner and outer leaflets undergo exchange (so-called 'flip-flop')^{12,13}. Another condition that induces a membrane flip-flop is apoptosis¹⁴, which may result at inflammatory sites following stimulation of cells with cytokines such as TNF.

Pancreatic PLA₂, which is closely related to sPLA₂ in terms of structure and function^{15,16}, is not able to hydrolyze efficiently the phospholipids of the outer membrane leaflet of normal intact cells because of their tight packing, but easily hydrolyzes those of the inner leaflet¹⁷. Recent studies have suggested a similar specificity for sPLA₂ (Refs 18, 19). Thus, sPLA₂ is only able to react with phospholipids in the outer leaflet of normal cells if they have undergone a flip-flop from the inner leaflet (Fig. 1). It is possible that this unique ability of sPLA₂ to discriminate normal from flip-flopped membranes allows the proinflammatory phase of the APR to target its

Fig. 1. Hypothetical role of secretory phospholipase A_2 (sPLA $_2$) and C-reactive protein (CRP) in promoting phagocytosis of injured cells during the early phase of the acute phase response (APR). (a) Phosphatidylserine (PS) and phosphatidylethanolamine (PE) (both shown as red dots) are only present in the inner leaflet (lower in the figure) of the phospholipid bilayer; this is an energy-dependent process requiring adenosine triphosphate (ATP). (b) The phospholipids of membranes of 'injured' cells may exchange between inner and outer leaflets ('flip-flop'), for example as the consequence of low intracellular ATP levels. (c) Flip-flopped membranes, but not normal membranes, are susceptible to hydrolysis by sPLA $_2$, which generates lysophosphatidylcholine (lyso-PC) in the outer leaflet. (d) The presence of lyso-PC in the outer leaflet creates binding sites for CRP, which results in activation of complement via the classical pathway. Activated complement attracts and activates neutrophils, which internalize the flip-flopped cell by phagocytosis. Cytokines, particularly IL-6, produced by macrophages and other cells in the injured tissue induce the synthesis of sPLA $_2$ and CRP by the liver.

effects to metabolically compromised cells (notably, these cells do not need to be irreversibly injured). After interaction with sPLA $_2$, cells are left with an increased proportion of lysophospholipids (phospholipids that have lost free fatty acids at the sn-2-ester bond) in the outer membrane leaflet. This modification, together with the presence of PS and PE, disturbs the packing of the phospholipids and generates binding sites for CRP in the outer leaflet.

CRP

CRP belongs to the pentraxin family of proteins, which are characterized by the presence of a basic structure of five identical, non-covalently bound subunits, arranged in cyclic symmetry. Initially, CRP was recognized by its ability to precipitate C-polysaccharide of pneumococci¹⁸. However, CRPs from some species are not endowed with this property²³; thus, it is doubtful whether binding to C-polysaccharide is the main function of CRP *in vivo*. Various other *in vivo* functions of CRP have been proposed, such as modulation of the function of immune cells. For example, CRP is able to induce the synthesis of anti-inflammatory cytokines by macrophages²¹, which may explain why administration of CRP under some experimental conditions can protect animals against inflammatory stimuli²². One *in vitro* function consistently linked to CRP is its ability to activate complement via the classical pathway^{22,24}. This probably results from the polymerization of CRP onto cells or bacteria, since chemical polymers of CRP also activate complement²⁵. As discussed below, recent *in vivo* work also supports the concept of CRP-mediated activation of complement, and it is possible that this represents the major function of CRP *in vivo*.

Localization in inflamed tissues

Early immunohistochemical studies suggested an active role for CRP in inflammatory conditions since it localizes in inflamed tissues^{26,27}. However, recent metabolic studies indicate that, at least in

humans, the localized fraction is likely to be low²⁸. CRP is capable of binding to artificial phospholipid bilayers provided that they contain a substantial amount of lysophosphatidylcholine (lyso-PC)²⁹. Therefore, CRP is probably able to bind to cells containing sufficient amounts of lyso-PC in the outer leaflet of their membranes. In agreement with this, CRP has been shown to bind to erythrocytes treated with snake PLA $_2$ (Ref. 30), an enzyme that potentially hydrolyzes phospholipids in the outer leaflet of cells, even in the absence of a membrane flip-flop. Thus, in inflamed tissues, it can be proposed that binding of CRP initially occurs to cells containing a significant proportion of lysophospholipids in the outer leaflet of their membrane due to the activity of sPLA $_2$ (Fig. 1). In addition, some binding of CRP might occur to cells with a flip-flopped membrane in the absence of significant amounts of lyso-PC (Refs 31, 32). Once bound, CRP induces complement activation via the classical pathway, which in turn triggers the influx of neutrophils (by generating C5a) and enhances phagocytosis of the cells that have bound CRP and complement³¹.

It is to be noted that the initial complement activation induced by CRP can be amplified by an autocatalytic mechanism: insertion of pore-forming proteins such as C9 into the membranes causes further flip-flop³¹ which, particularly in the presence of sPLA $_2$, creates additional binding sites for CRP (Ref. 32). This additional CRP in turn enhances complement activation, and so on. This autocatalytic CEF-mediated mechanism allows high concentrations of lysophospholipids to be generated locally in the outer leaflet of the membrane, even when the initial flip-flop was only moderate. These elevated concentrations not only provide more binding sites for CRP, but also hamper diffusion of lysophospholipids out of the membrane by favoring micelle formation.

Other pentraxins

Serum amyloid P component (SAP), another pentraxin, can bind to PE (Ref. 34) and activate complement³⁵. Since PE mainly occurs in

the inner leaflet of cell membranes, SAP may also promote complement activation by cells that have undergone a flip-flop. CRP and SAP are both found in many vertebrates and invertebrates¹. Remarkably, in most species, only one of these two proteins is an APP, whereas levels of the other remain constant during the APR. As described above, CRP is an APP in humans, whereas SAP is not¹. However, circulating levels of the latter are higher than those of CRP. Thus, it can be speculated that, under noninflammatory conditions (i.e. conditions where not too many cells are injured at the same time), the non-APP pentraxin is involved in the removal of cells with flip-flopped membranes, such as apoptotic cells resulting from the physiological regulation of cell growth and differentiation. Under conditions where many cells simultaneously undergo a flip-flop, such as during inflammatory reactions, the non-APP pentraxin is no longer able to mediate the removal of all these cells efficiently and, under the influence of locally produced inflammatory cytokines, the liver starts to synthesize large amounts of another pentraxin (the APP pentraxin) to compensate. Moreover, both APP and non-APP pentraxins not only bind to phospholipids, but also to nuclear material such as chromatin, histones and small nuclear ribonucleoprotein particles^{26,27}. Thus, either protein may also be involved in clearance of nuclear material originating from cells lysed by complement.

Evidence for CRP-mediated activation of complement *in vivo*

Activation of the classical complement pathway has been shown in patients receiving immunotherapy with high doses of IL-2 (Refs 38–40). These observations demonstrate the existence of a cytokine-mediated activation of complement *in vivo*, although its molecular mechanism is unclear. Vachino and co-workers⁴¹ observed that complement activation during IL-2 therapy was associated with the fixation of CRP to circulating lymphocytes, suggesting that CRP was responsible for the activation of complement induced by IL-2 treatment. Observations in baboons suffering from sepsis also point to the existence of CRP-mediated complement activation. In this model, complement is activated in a biphasic pattern: an early phase concomitant with the infusion of bacteria and probably resulting from direct activation of the system by the microorganisms; and a later phase that coincides with increasing levels of CRP (Ref. 42) and of sPLA₂ (C.E. Hack *et al.*, unpublished).

A recent immunohistochemical study investigated the localization of CRP and C3- and C4-activation fragments in the infarcted myocardium of 17 patients that had deceased in the acute phase of myocardial infarction⁴³. In all patients (except one with an infarction of less than 12 h duration), deposits of CRP were shown in the infarcted but not in the healthy myocardium. Moreover, in all cases, CRP was found to be co-localized with activated C3 and C4. This implies that the fraction of CRP deposited in inflamed tissues, although maybe low²⁸, is sufficient to activate complement.

CRP-mediated activation of complement *in vivo* might also be detected by the presence of specific circulating activation products. Sensitive assays specific for complexes comprising C4b/d or C3b/d

Fig. 2. Ability of C-reactive protein (CRP) to stimulate complement activation *in vitro*. EDTA-treated plasma was recalcified (to allow complement activation) and supplemented with CRP to a final concentration of 50 $\mu\text{g ml}^{-1}$ (green bars) or not (red bars). One volume of aggregated IgG (AgglgG; 3 mg ml^{-1}), lyso-phosphatidylcholine (lyso-PC; 400 $\mu\text{g ml}^{-1}$) or veronal buffered saline (VBS) was added, and the mixtures were incubated for 60 min at 37°C. The amount of C3b/d generated was then measured⁴⁹. Note that AgglgG-induced activation is independent of CRP levels, whereas lyso-PC-induced activation is dependent on CRP.

covalently bound to CRP have been developed⁴⁴. *In vitro* experiments in which serum containing elevated levels of CRP was incubated with various non-CRP activators of complement (e.g. aggregated IgG or *Escherichia coli* bacteria), and *in vivo* observations in patients receiving the monoclonal antibody OKT3 (OKT3 bound to lymphocytes activates the classical pathway), reveal that these complexes are only formed during CRP-mediated activation of complement, and not as innocent bystanders during complement activation by other activators, even in the presence of high CRP levels⁴⁴. Thus, elevated circulating levels of CRP-complement complexes reflect CRP-mediated activation of complement *in vivo*. Circulating levels of these complexes markedly increase in patients during treatment with IL-2 and this coincides with an increase in complement activation products such as C3a (G.-J. Wolbink *et al.*, unpublished).

Together, these data indicate that CRP is probably involved in the activation of complement in some clinical conditions; however, they do not provide information as to the ligand for CRP. The addition of lyso-PC to plasma containing increased levels of CRP provides a potent stimulus for CRP-mediated complement activation *in vitro* (Fig. 2), illustrating that lyso-phospholipids provide a suitable ligand for CRP. In all conditions where increased levels of CRP-complement complexes have been observed (e.g. IL-2 therapy, sepsis, major surgical procedures), marked increases of sPLA₂ were also shown. Furthermore, serial observations in patients receiving IL-2 (Ref. 45) indicate that complement activation does not occur

until both sPLA₂ and CRP start to increase (6–8 h after the bolus injection). These observations all agree with the hypothesis that CRP binds to cells via lyso-PC generated in the outer leaflet of the cell membrane by sPLA₂.

Is the amount of lyso-PC in membranes following hydrolysis by PLA₂ too low to provide sufficient fixation sites for CRP (at least 10% lyso-PC would be needed²³)? Recently, it was found that microvesicles in synovial fluid samples of patients with inflammatory arthritis may contain more than 10% lyso-PC (as well as substantial amounts of other lysophospholipids), which was claimed to be due to the action of sPLA₂ (Ref. 18). Therefore, it is reasonable to assume that, *in vivo*, sPLA₂ may generate enough lyso-PC, at least locally in flip-flopped membranes, to allow fixation of CRP.

Implications

CRP and sPLA₂ are highly conserved throughout evolution in vertebrates and may act together to enhance phagocytosis of metabolically compromised cells. Presumably, this mechanism has evolved to remove ischemic cells and tissue debris from wounds in order to allow quick repair of skin integrity. Inhibition of complement activation has been shown to reduce tissue necrosis, for instance after myocardial infarction²⁴. It is possible that the combined role of sPLA₂, CRP, complement and phagocytes in damaged tissue has not been fine-tuned to discriminate between reversibly and irreversibly injured cells. Rather, it discriminates between 'flip-flopped' and normal cells and, for the sake of speed (the sooner a skin defect is healed, the lower the risk of infection), reversibly injured cells are removed as well. In the skin, removal of reversibly injured cells has few detrimental effects since most cells present in this tissue are fibroblasts and epithelial cells, which easily proliferate. However, in organs mainly comprising cells that proliferate slowly, removal of reversibly injured cells by the APR may contribute to tissue damage. Thus, the clinical correlations of sPLA₂, CRP, complement activation and IL-6, observed in a number of diseases including sepsis,

arthritis and myocardial infarction, may reflect a basal pathogenic mechanism contributing to inflammation and tissue damage.

Finally, a number of APPs produced during the APR are capable of inhibiting the recruitment of neutrophils, the formation of their agonist²⁵, or the biological effects of toxic products released by these cells (Table 1). These APPs thus counteract the proinflammatory effects exerted by sPLA₂, CRP, complement and neutrophils, which may explain the finding that, under certain conditions, animals undergoing an APR are protected against inflammatory stimuli²⁶. These anti-inflammatory APPs may, therefore, serve to limit tissue damage induced by the proinflammatory phase of the APR.

Erik Hack and Cees-Jan Wolbink are at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and the Laboratory for Clinical and Experimental Immunology, University of Amsterdam, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands; Erik Hack is also at the Dept of Internal Medicine, Free University Hospital, Amsterdam, The Netherlands; Caspar Schalkwijk and Henk van den Bosch are at the Centre for Biomembranes and Lipid Enzymology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands; Han Spreijer and Willem Hermans are at the Cardiovascular Research Institute Maastricht, University of Maastricht, P. Debyealaan 25, 6202 AZ Maastricht, The Netherlands.

References

1. Stadnyk, A.W. and Gauldie, J. (1991) *Immunol. Today* 12, A7–A12
2. Baumann, H. and Gauldie, J. (1994) *Immunol. Today* 15, 74–80
3. Steel, D.M. and Whitehead, A.S. (1994) *Immunol. Today* 15, 61–68
4. Rodl, H., Schlag, G., Schlessner, A. and Davies, J. (1993) *Am. J. Physiol.* 264, H2119–H2123
5. Pruzanski, W. and Vadas, P. (1991) *Immunol. Today* 12, 143–146
6. Pfeilschifter, J., Schalkwijk, C., Briner, V.A. and van den Bosch, H. (1993) *J. Clin. Invest.* 92, 2516–2523
7. Glaser, K.B., Mobilia, D., Chang, J.Y. and Senko, N. (1993) *Trends Pharmacol. Sci.* 14, 92–98
8. van der Vusse, G.J., Glatz, J.P.C., Stam, H. and Reneman, R.S. (1992) *Physiol. Rev.* 72, 861–940
9. van den Bosch, H. (1980) *Biochim. Biophys. Acta* 604, 191–246
10. Crowl, R.M., Stoller, J.T., Conroy, R.R. and Stoner, C.R. (1991) *J. Biol. Chem.* 266, 2647–2651
11. Nevalainen, T.J. (1993) *Cell. Chem.* 39, 2453–2459
12. Zachowski, A. (1993) *Biochem. J.* 294, 1–14
13. Higgins, C.F. (1994) *Cell* 79, 393–395
14. Martin, S.J., Reutelingersperger, C.P.M., McMahon, A.J. et al. (1995) *J. Exp. Med.* 182, 1545–1556
15. Dennis, E.A. (1994) *J. Biol. Chem.* 269, 13057–13060
16. Ransac, S., Aarsman, A.J., van den Bosch, H., Gancet, C., de Haas, G.H. and Verger, R. (1992) *Eur. J. Biochem.* 204, 793–797
17. Demel, R.A., Goutas, van Kessel, W.S.M., Zwaal, R.F.A., Roelofs, B. and van Deenen, L.L.M. (1975) *Biochim. Biophys. Acta* 406, 97–107
18. Fourcade, O., Simon, M.F., Viode, C. et al. (1995) *Cell* 80, 919–927
19. Ransac, S., Aarsman, A.J., van den Bosch, H., Gancet, C., de Haas, G.H. and Verger, R. (1992) *Eur. J. Biochem.* 204, 793–797
20. Balz, M.L., de Beer, F.C., Feinstein, A. et al. (1982) *Ann. New York Acad. Sci.* 389, 49–75
21. Tilg, H., Vannier, E., Vachino, G., Dinarello, C.A. and Mier, J.W. (1993)

1. *Exp. Med.* 178, 1629-1636
- 22 Heuertz, R.M., Piquette, C.A. and Webster, R.O. (1993) *Am. J. Pathol.* 142, 319-328
- 23 Volanakis, J.E. (1982) *Ann. New York Acad. Sci.* 389, 235-249
- 24 Kilpatrick, J.M. and Volanakis, J.E. (1991) *Immunol. Rev.* 10, 43-53
- 25 Jiang, H., Rubey, F.A. and Gewurz, H. (1992) *J. Exp. Med.* 175, 1373-1379
- 26 Kushner, L., Rakita, L. and Kaplan, M.H. (1993) *J. Clin. Invest.* 92, 286-292
- 27 Kushner, L. and Kaplan, M.H. (1991) *J. Exp. Med.* 174, 961-974
- 28 Vigushin, D.M., Pepys, M.B. and Hawkins, P.N. (1993) *J. Clin. Invest.* 91, 1351-1357
- 29 Volanakis, J.E. and Wirtz, K.W.A. (1979) *Nature* 281, 155-157
- 30 Narkates, A.J. and Volanakis, J.E. (1982) *Ann. New York Acad. Sci.* 389, 172-182
- 31 Fattal, E., Nir, S., Parente, R.A. and Szoka, F.C., Jr (1994) *Biochemistry* 33, 6721-6731
- 32 Li, Y.-P., Mold, C. and Du Clos, T.W. (1994) *J. Immunol.* 152, 2995-3005
- 33 Mold, C., Du Clos, T.W., Nakayama, S., Edwards, K.M. and Gewurz, H. (1982) *Ann. New York Acad. Sci.* 389, 251-259
- 34 Schwalbe, R.A., Dahlback, B., Coe, J.E. and Nelsestuen, G.L. (1992) *Biochemistry* 31, 4907-4915
- 35 Ying, S.-C., Gewurz, A.T., Jiang, H. and Gewurz, H. (1993) *J. Immunol.* 150, 169-176
- 36 Pepys, M.B., Booth, S.E., Tennent, G.A., Butler, P.J.G. and Williams, D.G. (1994) *Clin. Exp. Immunol.* 97, 152-157
- 37 Hicks, I.S., Saunero-Nava, L., Du Clos, T.W. and Mold, C. (1992) *J. Immunol.* 149, 3689-3694
- 38 Thijs, L.G., Hack, C.E., Strack van Schijndel, R.J.M. et al. (1989) *Lancet* ii, 395
- 39 Thijs, L.G., Hack, C.E., Strack van Schijndel, R.J.M. et al. (1990) *J. Immunol.* 144, 2419-2423
- 40 Baars, J.W., Hack, C.E., Wagstaff, J. et al. (1992) *Br. J. Cancer* 65, 96-101
- 41 Vachino, G., Gelfand, J.A., Atkins, M.B., Tumerius, J.D., Demchak, P. and Mier, J.W. (1991) *Blood* 78, 2505-2513
- 42 de Boer, J.P., Creasey, A.A., Chang, A. et al. (1993) *Infect. Immun.* 61, 4293-4301
- 43 Lagrand, W.K., Niessen, H.W.M., Wolbink, G.J. et al. *Circulation* (in press)
- 44 Wolbink, G.J., Brouwer, M.C., Buysmann, S., ten Berge, R.J.M. and Hack, C.E. (1996) *J. Immunol.* 157, 473-479
- 45 Wolbink, G.J., Schellevis, C., Baars, J., Wagstaff, J., van den Bosch, H. and Hack, C.E. (1994) *Intensive Care Med.* 20, 566
- 46 Maroko, P.R., Carpenter, C.B., Chiariello, M. et al. (1978) *J. Clin. Invest.* 61, 661-670
- 47 de Graaf, T.W., van der Steet, M.E., Anbergen, M.G. and van Dijk, W. (1993) *J. Exp. Med.* 177, 657-666
- 48 Alcorn, J.M., Fierer, J. and Chofiker, M. (1992) *Hypothal.* 15, 122-129
- 49 Wolbink, G.J., Bollen, J., Baars, J.W. et al. (1993) *J. Immunol. Methods* 163, 67-76

The function of costimulatory molecules and the development of IL-4-producing T cells

William C. Gause, Mark J. Halvorson, Pin Lu, Rebecca Greenwald, Peter Linsley, Joseph F. Urban and Fred D. Finkelman

The development of IL-4-producing effector T cells during a type 2 immune response requires signaling through B7 ligands, but probably not CD40 ligands. Here, William Gause and colleagues review the still controversial role of CD28 and CTLA-4 on T cells, and B7-1 and B7-2 on antigen-presenting cells, and provide a model to incorporate recent findings.

immune response *in vivo* could aid the development of therapies directed at induction of T-cell unresponsiveness in the treatment of allergy, autoimmune disease and transplantation.

Costimulation in type 1 and 2 responses

Effector CD4⁺ Th cells have been generally subdivided into two groups depending on their cytokine profile: Th1 cells secrete interferon γ (IFN- γ), lymphotxin and interleukin 2 (IL-2); whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-9 and IL-10. The

T-cell activation leading to cytokine production and helper effector function requires a signal transduced through the antigen-specific T-cell receptor (TCR) as well as a second signal transduced by a costimulatory molecule. Costimulatory molecules may influence T-cell differentiation quantitatively, by determining the extent of T-cell activation, and qualitatively, by influencing the cytokines that are produced by T helper (Th) cells once they become activated. Understanding the effects of costimulatory molecules on Th-cell activation during the course of an