

For example, during aging, levels of IL-6 increase, levels of dehydroepiandrosterone decrease, the cortisol:ACTH ratio decreases (more evident in women than in men) and a shift towards Th2-type responses is observed (R.H. Straub).

#### Conclusion

This conference reflected a growing recognition that a fundamental knowledge and understanding of neuroendocrine interactions with the immunological and nervous systems need to be further acquired and related directly to basic and clinical aspects of rheumatic diseases. Clinically productive research on these complex mechanisms necessitates collaboration between investigators from many diverse specialities and countries. This conference provided an excellent basis for this.

#### References

- 1 Bijlsma, J.W.J. *et al.* (1999) The neuroendocrine immune basis of rheumatic diseases. *Immunol. Today* 20, 298–301
- 2 Straub, R.H. and Cutolo, M. (2001) Involvement of the hypothalamic–pituitary–adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis. *Arthritis Rheum.* 44, 493–507
- 3 Masi, A. *et al.* (2000) Neuroendocrine mechanisms in rheumatic diseases. *Rheum. Dis. Clin. North Am.* 26, 693–1017
- 4 Cutolo, M. *et al.* (2001) Abstracts NEI conference. *Clin. Exp. Rheumatol.* 19, 598–616
- 5 Merke, D.P. *et al.* (2000) Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *New Engl. J. Med.* 343, 1362–1368
- 6 van Everdingen, A.A. *et al.* Low-dose prednisone therapy for patients with early active rheumatoid arthritis: clinical efficacy, disease-modifying properties and side-effects. A double-blind placebo-controlled clinical trial. *Ann. Intern. Med.* (in press)

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## Complement in inflammatory tissue damage and disease

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The Complement-Associated Diseases, Animal Models and Therapeutics Workshop was held in Santorini, Greece from 10–14 October 2001.

During the past ten years, it has become evident that the complement system is an important mediator of inflammatory tissue damage in several diseases. Knockout animal models and novel agents that block complement-mediated effects have been developed to study the mechanisms of complement-mediated disease processes. For example, the tissue damage resulting from ischemia–reperfusion (IR) injury is largely owing to complement, and therapy with complement inhibitors is approaching the clinic.

#### A double-edged sword

The complement system (Fig. 1) is part of the host defense response. It has several biological effects, most of which contribute to the inflammatory reaction, mainly by the activation of cells such as leukocytes and endothelial cells. Patients with genetic complement deficiencies, particularly of the classical pathway, have an increased susceptibility to systemic lupus erythematosus (SLE)-like diseases, suggesting a beneficial role for complement in the induction of tolerance and clearance of immune complexes.

However, in SLE and other autoimmune diseases, there is a pathologically increased activation of complement, which might enhance the inflammatory reaction. Studies of knockout (KO) mice and specific complement inhibitors have shed light on this apparent contradiction. On the one hand, C1q-KO mice have defects in the phagocytosis of apoptotic cells, which are suggested to be the major source of autoantigens in SLE [1]. On the other hand, specific inhibition of C5 ameliorates the disease process in NZB/WF1 mice, a model of SLE [2]. Thus, an intact complement system is required not only for protection against infection, but also, for maintaining the internal inflammatory homeostasis. However, improper, enhanced or uncontrolled complement activation is disadvantageous for the host.

#### Keeping control

Complement activation is controlled carefully by several fluid-phase and membrane-bound inhibitors (Fig. 1). The need for keeping the complement system under control is illustrated by the fact that there are as many regulators as ordinary components of the cascade, and deficiency of any of the regulatory proteins is associated with substantially disturbed homeostasis. Human genetic

deficiency of C1 inhibitor (hereditary angioedema) is a serious condition involving life-threatening edema induced by capillary leakage. Factor-H deficiency leads to the uncontrolled activation of C3 and serious kidney disease. Somatic mutations in the phosphatidylinositol glycan class A (PIG-A) gene that impair synthesis of the glycosylphosphatidylinositol anchor required for membrane insertion of decay-accelerating factor (DAF) and CD59 lead to paroxysmal nocturnal hemoglobinuria, owing to the increased sensitivity of red blood cells to lysis. Several complement-regulator-deficient mice have been generated recently [3–5]. DAF-deficient mice have increased susceptibility to nephrotoxic serum nephritis [6] and also, deficiency of DAF exacerbates autoimmune disease in MRL/lpr mice, another model of human SLE (W.C. Song, Philadelphia, PA, USA). The increased sensitivity to nephrotoxic serum nephritis was confirmed in a second line of DAF KO mice (E. Medof, Cleveland, OH, USA). Red blood cells from a CD59-KO mouse were much more sensitive than those from wild-type mice to complement-induced lysis [5] and the CD59-KO mouse had exacerbated symptoms in induced models of arthritis and demyelination (B.P. Morgan, Cardiff, UK). The increased

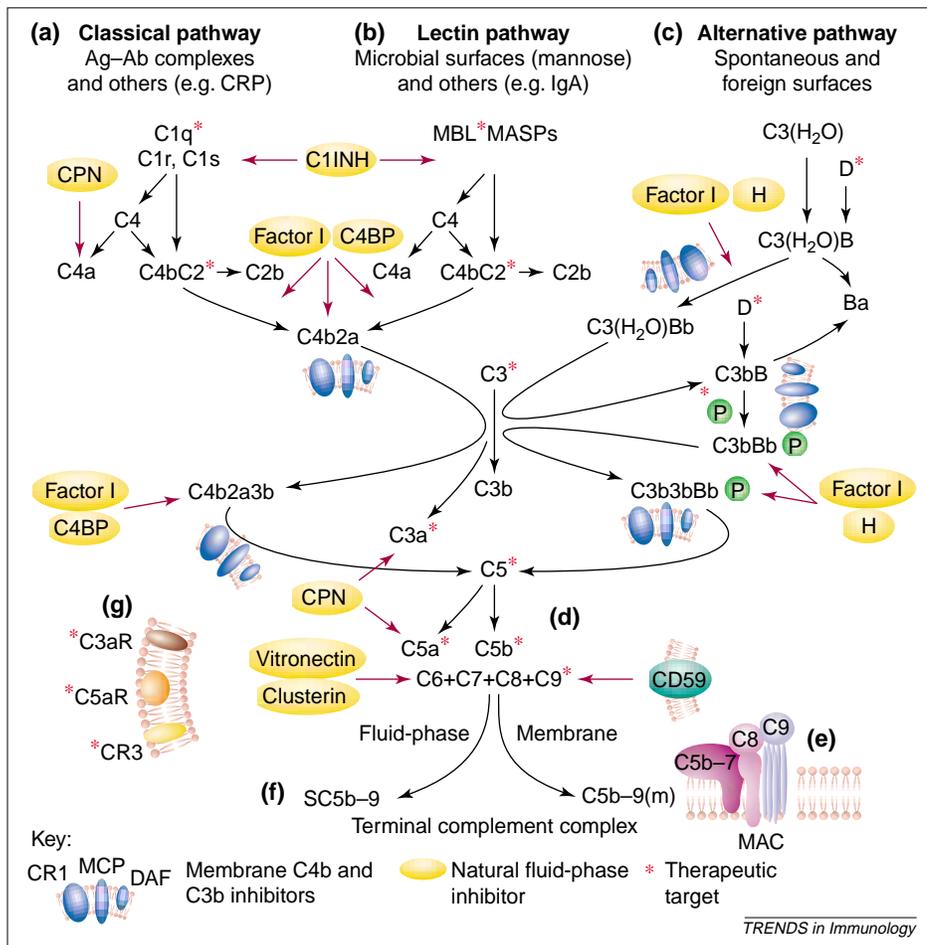


Fig. 1. The complement system comprises >30 proteins, which act together in a specific manner to protect the host against invading organisms. (a) The classical pathway is activated when natural or elicited antibodies (Abs) bind to antigen (Ag), or by other agents, such as C-reactive protein (CRP). C1q triggers the serine proteases C1r and C1s, the latter cleaving C4 to C4b, which exposes a specific binding site for C2. Then, C1s cleaves C2, and the resulting C3 convertase, C4b2a, cleaves C3 to C3b to form the C5 convertase C4b2a3b. Splitting of C5 to the highly potent anaphylatoxin C5a and the C6-binding fragment C5b is the last enzymatic step in the cascade. (b) Activation of the lectin pathway is initiated by mannose-binding lectin (MBL) recognizing mannose on bacteria, by IgA and probably, by structures exposed on damaged endothelium. MBL is homologous to C1q and triggers the MBL-associated serine proteases (MASPs), of which three forms (MASP1, MASP2 and MASP3) have been described. Downstream activation of the lectin pathway is virtually identical to classical-pathway activation, forming the same C3 and C5 convertases. (c) The alternative-pathway activation mechanisms differ from those of the classical and lectin pathways. Under normal physiological conditions, the C3 molecule undergoes a low-grade spontaneous hydrolysis of its internal thiol-ester and, thereby, binds factor B, which is cleaved by factor D to form a C3 convertase containing the whole C3 molecule [C3(H<sub>2</sub>O)Bb]. Then, this complex cleaves C3 to C3a and C3b. The latter binds factor B, which is cleaved by factor D to form the second alternative pathway C3 convertase C3bBb. Properdin (P), the only regulator of complement that amplifies activation, binds to C3bBb and stabilizes this complex, which then cleaves C3 and binds C3b to form the C5 convertase C3b3bBbP. This cleaves C5 in the same manner as the C5 convertase of the classical and lectin pathways. (d) The terminal pathway proceeds in the same manner, irrespective of the initial pathway activation, by the assembly of C7 to C5b6, forming an amphiphilic complex able to insert into a lipid membrane. (e) One C5b-7 moiety binds one C8 and one or more C9 molecules, creating a physical pore that penetrates the membrane [C5b-9(m) or membrane attack complex (MAC)], leading to transmembrane leakage and subsequent cell activation or, more infrequently, lysis. (f) If the activation occurs in the fluid-phase and there is no membrane present, the C5b-7 complex binds to vitronectin and clusterin (fluid-phase regulators of the terminal pathway) and thus, retains its hydrophilic properties. Final assembly of soluble C5b-9 (SC5b-9), the second form of the terminal complement complex, occurs by binding of C8 and C9. Complement activation is regulated strictly by inhibitory proteins. In the fluid phase, C1 inhibitor (C1INH) controls C1r, C1s and MASPs, whereas carboxypeptidase N (CPN) inactivates the anaphylatoxins C5a, C3a and C4a by removing their terminal arginine. Factor I cleaves and inactivates C4b and C3b, using C4b-binding protein (C4BP) as a cofactor in the classical and lectin pathways and factor H in the alternative pathway. The membrane regulators complement receptor 1 (CR1/CD35), membrane cofactor protein (MCP/CD46) and decay accelerating factor (DAF/CD55) regulate complement activation by acting as cofactors for factor-I-mediated cleavage of C4b and C3b (CR1 and MCP), or accelerating the decay of the C3 and C5 convertases (CR1 and DAF). CD59, also a membrane regulator, prevents the binding of C9 to the C5b-8 complex in the terminal pathway. CR1 and MCP are transmembrane proteins, whereas DAF and CD59 attach to the cell membrane through a glycosylphosphatidylinositol anchor. (g) Many of the biological effects resulting from complement activation are mediated by membrane receptors, such as the receptors for C1q, C3a (C3aR), C5a (C5aR) and iC3b (CR3/CD11b-CD18). Activated complement is a double-edged sword, with undesired effects in many conditions. Thus, various reagents with potential therapeutic applications have been developed to target complement activation and function (targets are indicated by red asterisks).

sensitivity of red blood cells to classical-pathway complement-mediated lysis was demonstrated also in another line of CD59-KO mice (W.C. Song). Also, W.C. Song has created a DAF/CD59 double-KO mouse and demonstrated marked synergism of protection between DAF and CD59 on red blood cells. Interestingly, DAF/CD59 double-KO mice do not suffer from hemolytic anemia, suggesting that complement-receptor-1-related protein  $\gamma$  (Cr $\gamma$ ), which is the only membrane regulator remaining on DAF/CD59-deficient mouse erythrocytes, might be sufficient for inhibiting the spontaneous activation of complement on these cells. Future studies of these regulator-deficient mice are expected to shed new light on the role of complement in inflammatory tissue damage.

**Adaptive immunity and hypersensitivity**  
The link between complement and adaptive immunity, in terms of enhancing antigen presentation and antibody production, has been established using C3- and C4-deficient mice [7] and mice lacking complement receptor 1 (CR1) and/or CR2 on their B cells [8]. The binding of antigen to C3d and ligation of CR2 (CD21) with CD19 are essential events in this link [9]. There are several therapeutic possibilities arising from the interaction between complement and adaptive immunity, including the modulation of vaccines and induction of tolerance. A direct role for complement in T-cell-mediated contact hypersensitivity was suggested, because this reaction is impaired in C5a receptor (C5aR)-deficient mice [10]. These data were confirmed using a C5aR antagonist, which abrogated the early (2–4 hour) phase and attenuated the late (24 hour) phase of contact hypersensitivity (J. Koehl, Hannover, Germany). Interestingly, whereas the C5a–C5aR interaction seems to be essential for contact hypersensitivity (and, indeed, for the majority of complement-mediated inflammatory reactions), the C3aR seems to be the most important in hypersensitivity of the upper airways. For example, a pathogenic role for C3a in asthma was reported recently [11, 12]. These observations emphasize the need for differentiation of the role of individual complement components and their specific targeting in various pathophysiological conditions.

### Improper tissue damage – the ischemia–reperfusion injury

Since Weisman *et al.* [13] demonstrated, a decade ago, a marked reduction in the tissue damage owing to myocardial infarction using recombinant soluble CR1, several studies have confirmed that complement is an important mediator of tissue damage in IR injury. C3-KO and Ig-deficient mice were protected against skeletal muscle IR injury, suggesting a role for naturally occurring antibodies and complement in the tissue damage in this model [14]. Recently, the lectin pathway has been proposed to be important for the generation of myocardial infarction lesions in rats [15]. Thus, the initial pathway-activation mechanisms involved in IR injury might vary depending on the condition. This seems to be the case also for the effector mechanisms triggering the tissue damage. Terminal-pathway activation is essential for most IR injuries, but the relative roles of C5a and C5b–9 seem to vary. In a mouse renal IR model, the primary effect of complement was on the parenchymal cells and was completely dependent on C5b–9 [16]. Although C5b–9 might play some role in the local intestinal IR injury of mice also [17], C5a is probably the most important mediator of damage in this condition [18]. This view was supported by data showing that mice (S.D. Fleming, Silver Spring, MD, USA) or rats (T.V. Arumugum, Queensland, Australia) treated with a C5aR antagonist were protected against local intestinal IR injury and remote organ injury. In porcine myocardial IR injury, a C5aR antagonist reduced the infarct size markedly [19], whereas C5b–9 was the main mediator of myocardial damage in a model of rabbit hearts perfused with human serum [20]. Interestingly, the latter group showed that pre-treatment with sublytic doses of complement protected the heart from subsequent IR injury, by a C5a-mediated mechanism [21].

### Therapeutic strategies

Various recombinant human complement inhibitors have been developed, as well as monoclonal antibodies, synthetic peptides and peptidomimetics, which either block activation of a certain component, neutralize an activation fragment or antagonize a complement receptor. Different targets for the inhibition of the complement cascade are illustrated in Figure 1, and some of the most promising inhibitors are listed in Table 1.

**Table 1. Examples of therapeutic targets and inhibitors of the complement system<sup>a</sup>**

Target	Inhibitor <sup>b</sup>	Application	Refs
Classical pathway: C1q	Peptides	Xenotransplantation (animal)	[22]
Lectin pathway: MBL	Anti-MBL mAb	Myocardial IR injury (animal)	[15]
Classical and/or lectin pathway	C1 inhibitor	Numerous animal models and clinical trials	[23]
Alternative pathway: factor D	Anti-factor-D mAb	Cardiopulmonary bypass (animal)	[24]
Common pathway: C3	Recombinant sCR1 and peptides (e.g. compstatin)	Myocardial IR injury (animal)	[13]
		Acute lung injury (clinical)	[25]
		Xenotransplantation ( <i>ex vivo</i> )	[26]
		Heparin/protamin activation (animal)	[27]
Terminal pathway:			
C5	Anti-C5 mAb	Cardiopulmonary bypass (clinical)	[28]
C5a	Anti-C5a mAb	Septicemia (animal)	[29]
Complement receptors:			
C3aR	Peptide antagonist	LPS-induced airway neutrophilia (animal)	[30]
C5aR	Peptide antagonist	Endotoxic shock (animal)	[31]

<sup>a</sup>Abbreviations: IR, ischemia–reperfusion; LPS, lipopolysaccharide; mAb, monoclonal antibody; MBL, mannose-binding lectin; sCR1, soluble complement receptor 1.  
<sup>b</sup>Additional inhibitors that have emerged recently include soluble membrane cofactor protein (MCP), decay-accelerating factor (DAF), CD59 and conjugates thereof.

C1 inhibitor is a naturally occurring regulator of the classical and lectin pathways of complement activation, and in addition, it inhibits the kallikrein–kinin system, thereby reducing the formation of bradykinin. Purified C1 inhibitor obtained from human plasma has been used as a substitution therapy for patients with hereditary angioedema for several decades. Data from experimental IR injury studies highlight myocardial infarction as one of the most relevant conditions for anti-complement therapy. A phase I/II clinical trial using C1 inhibitor for this purpose was described (C.E. Hack, Amsterdam, The Netherlands). C1 inhibitor was administered to the patients after thrombolytic therapy and continued for two days, giving a two–threefold increase in physiological concentration. The size of the infarct, as measured by levels of troponin T and creatine kinase isoenzyme 2 (CK-MB), was reduced by 50% compared with historical controls. These promising results imply that a recombinant form of C1 inhibitor and placebo-controlled clinical studies are highly desired.

### '...uncontrolled complement activation is disadvantageous for the host.'

Targeting complement as a therapeutic goal requires detailed knowledge of the activation mechanisms and mediators responsible for inflammation, enabling optimal inhibitory treatment for the actual condition. Many mechanisms have been elucidated, particularly in IR injury,

but the field is still in its infancy. The main challenge for the future will be to balance the beneficial effects of inhibition of the complement cascade with the preservation of sufficient functional activity for microbial protection and tissue renovation.

### Acknowledgements

We apologize that, owing to space constraints, only a few of the excellent contributions to the meeting could be included in this report.

### References

- Taylor, P.R. *et al.* (2000) A hierarchical role for classical-pathway complement proteins in the clearance of apoptotic cells *in vivo*. *J. Exp. Med.* 192, 359–366
- Wang, Y. *et al.* (1996) Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8563–8568
- Sun, X. *et al.* (1999) Role of decay-accelerating factor in regulating complement activation on the erythrocyte surface as revealed by gene targeting. *Proc. Natl. Acad. Sci. U. S. A.* 96, 628–633
- Xu, C.G. *et al.* (2000) A critical role for murine complement regulator Crry in fetomaternal tolerance. *Science* 287, 498–501
- Holt, D.S. *et al.* (2001) Targeted deletion of the CD59 gene causes spontaneous intravascular hemolysis and hemoglobinuria. *Blood* 98, 442–449
- Sogabe, H. *et al.* (2001) Increased susceptibility of decay-accelerating-factor-deficient mice to anti-glomerular basement-membrane glomerulonephritis. *J. Immunol.* 167, 2791–2797
- Wessels, M.R. *et al.* (1995) Studies of group B streptococcal infection in mice deficient in complement component C3 or C4 demonstrate an essential role for complement in both innate and acquired immunity. *Proc. Natl. Acad. Sci. U. S. A.* 92, 11490–11494

- 8 Croix, D.A. *et al.* (1996) Antibody response to a T-dependent antigen requires B-cell expression of complement receptors. *J. Exp. Med.* 183, 1857–1864
- 9 Fearon, D.T. and Carroll, M.C. (2000) Regulation of B-lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu. Rev. Immunol.* 18, 393–422
- 10 Tsuji, R.F. *et al.* (2000) Early local generation of C5a initiates the elicitation of contact sensitivity by leading to early T-cell recruitment. *J. Immunol.* 165, 1588–1598
- 11 Humbles, A.A. *et al.* (2000) A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 406, 998–1001
- 12 Bautsch, W. *et al.* (2000) Cutting edge: guinea pigs with a natural C3a-receptor defect exhibit decreased bronchoconstriction in allergic airway disease: evidence for an involvement of the C3a anaphylatoxin in the pathogenesis of asthma. *J. Immunol.* 165, 5401–5405
- 13 Weisman, H.F. *et al.* (1990) Soluble human complement receptor type 1: *in vivo* inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 249, 146–151
- 14 Weiser, M.R. *et al.* (1996) Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J. Exp. Med.* 183, 2343–2348
- 15 Jordan, J.E. *et al.* (2001) Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. *Circulation* 104, 1413–1418
- 16 Zhou, W.D. *et al.* (2000) Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J. Clin. Invest.* 105, 1363–1371
- 17 Austen, W.G. *et al.* (1999) Intestinal ischemia–reperfusion injury is mediated by the membrane attack complex. *Surgery* 126, 343–348
- 18 Heller, T. *et al.* (1999) Selection of a C5a receptor antagonist from phage libraries attenuating the inflammatory response in immune complex disease and ischemia reperfusion injury. *J. Immunol.* 163, 985–994
- 19 Riley, R.D. *et al.* (2000) Recombinant human complement C5A receptor antagonist reduces infarct size after surgical revascularization. *J. Thorac. Cardiovasc. Surg.* 120, 350–358
- 20 Homeister, J.W. *et al.* (1992) Effects of complement activation in the isolated heart – role of the terminal complement components. *Circ. Res.* 71, 303–319
- 21 Tanhehco, E.J. *et al.* (2000) Sublytic complement attack reduces infarct size in rabbit isolated hearts: evidence for C5a-mediated cardioprotection. *Immunopharmacology* 49, 391–399
- 22 Fryer, J.P. *et al.* (2000) Synthetic peptides which inhibit the interaction between C1q and immunoglobulin and prolong xenograft survival. *Transplantation* 70, 828–836
- 23 Kirschfink, M. and Mollnes, T.E. (2001) C1-inhibitor: an anti-inflammatory reagent with therapeutic potential. *Expert Opin. Pharmacother.* 2, 1073–1084
- 24 Fung, M. *et al.* (2001) Inhibition of complement, neutrophil and platelet activation by an anti-factor-D monoclonal antibody in simulated cardiopulmonary bypass circuits. *J. Thorac. Cardiovasc. Surg.* 122, 113–122
- 25 Zimmerman, J.L. *et al.* (2000) Phase I trial of the recombinant soluble complement receptor 1 in acute lung injury and acute respiratory distress syndrome. *Crit. Care Med.* 28, 3149–3154
- 26 Fiane, A.E. *et al.* (1999) Compstatin, a peptide inhibitor of C3, prolongs survival of *ex-vivo*-perfused pig xenografts. *Xenotransplantation* 6, 52–65
- 27 Soulika, A.M. *et al.* (2000) Inhibition of heparin/protamine-complex-induced complement activation by Compstatin in baboons. *Clin. Immunol.* 96, 212–221
- 28 Fitch, J.C.K. *et al.* (1999) Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation* 100, 2499–2506
- 29 Czermak, B.J. *et al.* (1999) Protective effects of C5a blockade in sepsis. *Nat. Med.* 5, 788–792
- 30 Ames, R.S. *et al.* (2001) Identification of a selective nonpeptide antagonist of the anaphylatoxin C3a receptor that demonstrates antiinflammatory activity in animal models. *J. Immunol.* 166, 6341–6348
- 31 Strachan, A.J. *et al.* (2000) A new small molecule C5a receptor antagonist inhibits the reverse-passive arthus reaction and endotoxic shock in rats. *J. Immunol.* 164, 6560–6565

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## From A to Z on CpG

Arthur M. Krieg

The Second International Symposium on Activating Immunity with CpG Oligos was held at the Ritz Carlton in Amelia Island, FL, USA from 7–10 October 2001.

This meeting brought together scientists to discuss the latest developments in immune stimulation with CpG motifs from microbial DNA, ranging from the molecular biology of CpG recognition to the effects of CpGs in animal models and the first human clinical trials. For those unfamiliar with the field, the immune system is activated by microbial, but not vertebrate, DNA [1] due to unmethylated CpG dinucleotides (CpG motifs) [2,3] that act through a specific receptor, toll-like receptor 9 (TLR-9) [4,5].

#### CpG signaling

New results presented at the meeting provided a much clearer picture of the molecular mechanisms of the recognition of CpG motifs by TLR-9. S. Bauer (Munich, Germany) pointed out that TLR-9 contains two CXXC motifs (found in DNA methyltransferases) and a methyl CpG-binding domain (found in the methyl-CpG-binding protein). Bauer's experiments suggested that TLR-9 binds directly to CpG DNA, and the second CXXC motif of the mouse TLR-9 protein might modulate the signal strength of TLR-9, but not its binding specificity. The possibility of a second CpG-binding protein was raised by K. Stacey (Brisbane, Australia), who showed that replication protein A (RPA) forms a specific stable complex with CpG oligodeoxynucleotides

(ODNs). Moreover, RPA associates with DNA-PK, suggesting a potential link to its reported role in CpG signaling [6].

R. Medzhitov (New Haven, CT, USA) reported that Toll/interleukin-1-receptor-associated protein (TIRAP) is required for all lipopolysaccharide (LPS)-mediated, but not CpG-mediated, signaling events. Conversely, H. Wagner (Munich, Germany) showed that RAB5, a protein involved in endosome formation, is required for signal transduction by CpG DNA, but not LPS.

#### Expression patterns of TLR-9

There was broad agreement that only cells expressing TLR-9 [B cells and plasmacytoid dendritic cells (PDCs), but not resting macrophages in humans] can be activated directly by CpG DNA [7,8].