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Short communication

Innate immunity in the Aegean: ancient pathways for today's survival

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Abstract

A workshop on innate immunity that took place this past autumn in Fira, Santorini, as part of the Aegean Conferences, provided tantalizing evidence about the early origin and evolutionary conservation of humoral and cellular components of innate immunity from sponges, flies and sea squirts to man, uncovered mechanistic aspects of its fundamental role in defense against disease, as well as the serious consequences of misdirected responses, and revealed the untapped potential of novel therapeutic approaches. © 2002 Published by Elsevier Science Ltd.

1. Evolutionary aspects of innate immunity in invertebrates and vertebrates

That innate immunity preceded adaptive immunity in evolution has been supported by the presence of conserved signaling pathway components in organisms lacking the typical adaptive immunity of vertebrates [1]. The concept that innate immunity mechanisms were already operative before the proto/deuterostome evolutionary divergence is now supported by the application of subtractive techniques to the simplest multicellular organisms, such as marine sponges (Humphreys, University of Hawaii, Honolulu, USA), revealing the presence of homologs of 'vertebrate' innate immunity components as putative factors responsible for sponge allorecognition. Functional genomics approaches have also yielded novel information in the evolution of immune mechanisms. For the first time, a homologue (65% identity) of the mammalian nuclear factor of activated T-cells 5, a member of the Rel family of transcription factors, was identified in an invertebrate species (*Drosophila*) (Hultmark et al., Umeå Centre for Molecular Pathogenesis, Umeå University, Sweden). The ubiquitous expression and increase of the transcript in adult wild type flies after a bacterial challenge suggest that, like the mammalian counterpart, this protein is involved in the insect immune response. At the opposite end of the invertebrate phylogenetic tree, a diverse lectin repertoire is present in plasma and hemocytes of the protochordate *Clavelina picta*, a sea squirt, suggesting that despite the lack of the typical adaptive combinatorial mechanisms of vertebrates, these organisms display a certain diversity in self/non-self recognition (Vasta et al., University of Maryland Biotechnology Institute, Baltimore, USA). Further, the identification of a lectin with tandem carbohydrate domains similar to mannose-binding lectins (MBLs) and selectins, suggests that it may be an evolutionary precursor of the divergent vertebrate

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homologs. The finding of putative molecular partners (C3, MASP and galectins) of the tunicate MBL-like CRDs suggests that the lectin-mediated complement activation pathway is already present in tunicates. The evolutionary position occupied by the protochordates, bridging invertebrates and vertebrates, suggests that these organisms may express extant examples of the most advanced defense mechanisms that preceded the advent of immunoglobulins [2], while at the same time support the hypothesis that the lectin-mediated pathway for complement activation may have preceded the immunoglobulin-mediated pathway [3].

The diversification of the complement components C3 in fish may represent a critical stage in the evolution of both innate and adaptive defense mechanisms (Lambris et al., University of Pennsylvania, Philadelphia, USA). The appearance of complement predates the emergence of adaptive immunity [3,4]. In virtually all species analyzed to date, functionally active C3 exists as the product of a single gene. However, several fish, including trout, sea bream, medaka and carp possess C3 in various isoforms that are the products of different genes [4]. Trout and sea bream C3 isoforms have been shown to significantly differ in their binding efficiencies to various activating surfaces, and this specificity appears to be associated with structural differences within the regions of C3 that determine specificity for various surfaces. The observed structural and functional diversity of C3 in fish might serve to augment the number of potential pathogens that C3 can recognize. Thus, expansion of lectin (MBL-like) and complement C3 repertoires in organisms lacking (protochordates) or displaying less efficient (fish) adaptive immunity mechanisms suggests that the protective role of the MBL-mediated complement activation may have been critical in certain stages of evolution of the chordate lineages, at the time when adaptive immunity had not yet fully developed. The complexity of vertebrate innate immune systems may have been enhanced by genome duplications that took place prior or during the emergence of the vertebrate phyla. The presence of at least two copies of components of MHC in hagfish, an agnathan which lacks a bona fide adaptive immune system, suggests that this group had already gone through one genome duplication event (Kasahara et al., School of Advanced Sciences, Hayama, Japan). Whereas some components of innate immunity in vertebrate taxa may be ancient and substantially conserved, others, such as the killer cell immunoglobulin-like receptors (KIR) for determinants of classical MHC class I, are rapidly evolving in primates as a consequence of multiple pressures, possibly including 'catching up' with the evolution of their polymorphic ligands (Parham, Stanford University, Palo Alto, USA). Chimpanzees have KIR systems as complex as humans, but the relative number of receptor subclasses as well as the structure and specificity of these receptors are different in the two species. Extending these studies to other ape species should provide further proof to this intriguing proposal.

2. Humoral recognition and effector factors: lectins, complement activation and complement receptors

The finding that mammalian humoral non-self recognition/opsonic factors such as C-reactive protein (CRP) and the MBL, can also activate the complement cascade leading to direct killing of the pathogen through the membrane attack complex and other complement-mediated activities [3,4], has increased our interest in elucidating the detailed mechanisms of these pathways. New information about role(s) of serine proteases in triggering of the MBL pathway for complement activation was provided with regards to the proteolytic activities of the human MBL-associated serine proteases MASP-1 and MASP-2. Independent autoactivation of MASP-1 in the presence of proenzymic MASP-2 and viceversa, suggest autonomous behavior of these enzymes, and implies that individual MBL/MASP-1 and MBL/MASP-2 complexes may exist and function independently (Ambrus et al., Hungarian Academy of Sciences, Budapest, Hungary). Substantial evidence, however, suggest that MASP-2 is the critical component of the MBL/MASP complex(es) that mediates C4 and C2 cleavage, whereas MASP-1 only exhibits a weak C2-cleaving activity (Arlaud et al., Institut de Biologie Structurale, Grenoble, France). The role of CRP, an acute phase reactant in host defense against bacteria, was further confirmed (Szalai et al., University of Alabama at Birmingham, Birmingham, USA) by the use of CRP-transgenic mice challenged with

either *Streptococcus* or *Salmonella*. In early stages of bacterial infection, CRP protects the host by complement activation through the classical pathway, whereas in later stages, its protective effects are achieved rather by enhancement of the host's adaptive immune response.

Some aspects of recognition and regulatory mechanisms mediated by factor H have remained uncertain. Recombinant fragments, molecular modeling, mutagenesis and surface plasmon resonance were used by Meri et al. (Haartman Institute, Helsinki, Finland) to identify the critical sites of the factor H molecule that interact with C3b and surfaces rich in sialic acid or glycosaminoglycans (GAGS). Platelet-mediated phosphorylation of C3 may constitute another mechanism for regulation of complement since it potentiates binding of C3b to immune complexes and the interaction of immune complex-bound C3b with CR1 bearing cells (Nilsson et al., University Hospital, Uppsala, Sweden). The mechanisms of direct complement-mediated killing of Gram-negative bacteria by the membrane attack complex (C5b-9), and the means used by bacteria to become resistant have remained elusive. In the periplasmic space of an *Escherichia coli* cell C9 is converted from a protoxin to a toxin, independently of the pathway by which C9 enters the periplasm. Translocation into the periplasm may provide a novel model to identify bacterial killing mechanisms of other disulfide-linked proteins and to investigate bacterial complement-resistance (Esser et al., University of Missouri-Kansas City, USA).

Originally described as a high affinity receptor protein which binds to C1q, evidence has accumulated in support of gC1q-R being a multiligand binding protein which interacts with proteins of the intrinsic coagulation/bradykinin-forming cascade, and supporting the proposal that gC1q-R plays an important role in inflammation. Intriguing evidence was presented (Ghebrehiwet et al., State University of New York, USA) that gC1q-R can also serve as a receptor for bacterial proteins such as internalin, an invasion protein of *Listeria monocytogenes*, and Protein A, a multifunctional virulence protein of *Staphylococcus aureus*, suggesting that it mediates pathogenesis by these microorganisms. On the other hand, the phagocytic complement C1q receptor (C1qRp) in monocytes has been shown to be involved in the enhancement of phagocytosis of target cells opsonised with iC3b and immunoglobulins by monocytes. Results by McGreal et al. (Department of Medical Biochemistry, UWCM, Cardiff, UK) indicate that C1qRp is also present in CD8+ cells and cells expressing natural killer cell phenotype, suggesting that this receptor may not simply be involved in the enhancement of phagocytosis as first suggested, but may play a more general role in pathogen recognition and subsequent potentiation of cells involved in host defense. In the future, it will be important to determine whether crosslinking of C1qRp on these cells translates into an increased cytotoxic effect from NK cells. In a murine model, the homologous receptor is also present in basophilic cells, suggesting the possibility that it may mediate degranulation.

In understanding how complement activation may lead to adaptive immunity, a provocative explanation of how the combination of naturally occurring antibodies and complement may contribute to induce a primary immune response to non-self was contributed by Lutz (Institute for Biochemistry, ETH-Zentrum, Zurich, Switzerland). His hypothesis is based on the preferential binding of C3b to natural antibodies leading to immune complexes that bind to B cells, leaving only those specific for non-self epitopes to be stimulated for directed affinity maturation.

3. Signalling pathways and activation of innate and adaptive immune responses

Humoral mediators and signalling pathways for immunoactivation were topics in which multiple contributions revealed substantial recent progress. This also included regulatory aspects of great interest, such as the control of innate immune responses mediated by neuropeptides released within the lympoid organs upon antigen stimulation. New insight on the effects of the VIP and PACAP neuropeptides on macrophage function was provided by Doina Ganea (Rutgers University, Dept. Biological Sciences, Newark, NJ, USA). PACAP downregulates the expression of macrophage-derived pro-inflammatory agents such as IL-12, TNFα, IL-6 and NO, and enhance the production of the anti-inflammatory cytokine IL-10, both in vitro and in vivo. VIP/PACAP also

downregulate B7.1/B7.2 expression and reduce the macrophage costimulatory activity for T cells. Studies of the transduction pathways and transcriptional factors revealed that VIP/PACAP use a cAMP-independent and dependent pathways to modulate transcriptional factors, ultimately downregulating TNFα, IL12p40 and iNOS transcription, and upregulating IL-10 transcription. Thus, VIP and PACAP act as endogenous anti-inflammatory agents that contribute to the control of both innate and adaptive immunity. Also, the role(s) of cytokines in both innate and adaptive immunity were addressed. Interleukin-15, a pluripotent cytokine known to upregulate the cytocidal activity of lymphocytes, particularly of NK cells, was functionally characterized by exposing human peripheral blood leukocyte cultures to cytolytic (i.e. herpes simplex-1, HSV-1) and cell-immortalizing (i.e. Epstein-Barr virus, EBV) virus. Results clearly indicate that upregulation of IL-15 gene expression (leading to IL-15 protein production) is an early signal of the innate immune response to infectious agents (Menezes et al., Department of Microbiology & Immunology, University of Montreal, Montreal, Canada).

The contribution of Toll-like receptors to immune activation was addressed in several aspects. In mice, genetic evidence suggesting that Tlr4 is the LPS receptor was reported, but biochemical evidence that murine Tlr4 confers LPS responsiveness has not been demonstrated. NF-kB mediates one of major LPS-induced signaling pathways and COX-2 is selectively expressed in LPS-stimulated macrophages. However, there were contradicting reports regarding the role of NF-kB in LPS-induced COX-expression. Evidence supporting that Tlr4 mediates LPS-induced signaling and that LPS-induced COX-2 expression is in part mediated through NF-kB in a murine macrophage cell line (raw 264.7) was presented by Hwang and Rhee (Pennington Biomedical Research Center, La. State Univ., Baton Rouge, USA). Based on evidence indicating that vascular smooth muscle and endothelial cells within atherosclerotic lesions exhibit characteristics of active inflammatory response, Bourcier et al. (Brigham & Women's Hospital, Cardiovascular Division, Boston, USA) examined the expression and contribution of Toll-like receptors to activation of human vascular smooth muscle and endothelial cells, and found that both cell types express TLR4 in vitro, and can initiate proinflammatory signaling pathways in these cell types. They proposed that constitutive expression of TLR4 in normal vessels may serve a sentinel function in the vessel wall against injury or sepsis. Enhanced TLR4 expression by vascular cells within early and advanced lesions suggests that TLR4 participates in the pathogenesis of human atherosclerosis. New insight on the in vivo regulation and induction of innate immune defence mechanisms by Tlrs was obtained on a murine infection model in which the distribution and regulation of TLR4 and TLR2 in a subset of organs following experimental infection with a sub-lethal dose of *E. coli* K12 strain (Richter-Dahlfors et al., Karolinska Institute, Stockholm, Sweden). Gene expression showed organ-specific and time-dependent variation during the course of infection and significant differences were found between TLR2 and TLR4 synthesis, suggesting that TLR expression is differentially regulated after microbial challenge in order to optimize recognition and initiate innate immune defense mechanisms. Evidence was presented also that in primary epithelial cells, α-hemolysin, a RTX toxin secreted by uropathogenic strains of *E. coli*, induces a constant, low frequency intracellular oscillatory calcium response, which stimulates increased production of IL-6 and IL-8, probably due to frequency modulation of NF-kB expression. A novel role for α-hemolysin during the early stage of infection was proposed, as an inducer of second messenger response that fine-tunes gene expression into pathways leading to inflammatory responses.

Activation of polymorphonuclear neutrophils via occupation of the G protein-coupled receptors of chemotactic factors depends upon several signaling pathways prominent among which are those dependent on tyrosine phosphorylation events and on the production of phosphatidylinositol 3,4,5-trisphosphate. Interesting results on the potential involvement of the G protein-coupled PI 3kinase, p110γ, in the responses of human neutrophils to chemotactic factors and its relationship to the stimulation of the tyrosine phosphorylation cascade were discussed by Naccache et al. (Laval University, Ste-Foy, Québec). Their studies suggest that the sequence of events that lead from G protein-coupled receptors to the tyrosine phosphorylation cascades depends on p110γ and the recruitment of Tec family kinases.

New information—a 'red flag'—relevant to potential applications in the design of DNA vaccines was contributed by Ashman et al. (Department of Veterans Affairs, Iowa City, USA). Because bacterial DNA provides a danger signal for the mammalian immune system, these investigators tested oligonucleotides containing CpG motifs similar to those of bacterial DNA as immunization adjuvants, and identified inhibitory CpG oligonucleotides which specifically block effects of stimulatory CpG oligonucleotides in B cells. Thus, the very important finding is that the design of CpG-based vaccine adjuvants and plasmid vectors for DNA immunization must not only include stimulatory ODN sequences but avoid the inhibitory ones.

4. Natural killer and phagocytic cells: receptors and cell activation

A comprehensive review of NK cell effector functions and the specific receptors that mediate inhibition and cytotoxicity was presented by Moretta (Università degli Studi di Genova, Italy). Some NK receptors, such as KIRs, that mediate recognition of MHC-class I molecules on normal cells, and thus inhibition of cytolytic functions, have been known for some time. Others, such as ILT-2/LIR-1 and p49 NK, members of the Ig superfamily that display broad specificity for a number of HLA-class I alleles, have been more recently characterized. NK cell surface receptors (NCRs) that mediate NK cell cytotoxicity, however, are the newest to be identified. These include NKp46, NKp44 and NKp30, and although belonging to the Ig superfamily, their sequences failed to show any significant homology to any described proteins.

Intriguing information concerning a novel role for SH2-containing inositol phosphatase (SHIP) in the maintenance of homeostasis in the NK cell compartment was presented by Kerr (Moffitt Cancer Center, University of South Florida, Tampa, USA). SHIP is known to play a role in regulating the effector function of B lymphocytes and mast cells. Using a knockout mice approach, Kerr and collaborators have obtained evidence strongly suggesting that by influencing the surface expression of receptors, SHIP signaling would be a critical factor in the establishment and maintenance of the NK cell repertoire and their differentiation to cytolytic effector cells. New insight on the role(s) of NK cell subsets in the acute rejection of bone marrow cell (BMC) allografts was provided by Murphy (SAIC Frederick, NCI-FCRDC, Frederick, USA). Previous work by this lab has shown that the outcome of the transplant depends in part on the recognition, or lack thereof, between Ly-49 receptors on NK cell subsets and MHC present on the allografts. Activating and inhibitory receptor-bearing NK cell subsets (i.e. Ly-49D and Ly-49C, respectively) can work together in the rejection of H2d BMC by H2b mice. NK cell subsets bearing different inhibitory receptors (i.e. Ly-49A and Ly-49G2) can synergize in their ability to reject H2b BMC. Now, opposing NK cells have been demonstrated to mediate the acute rejection of BMC allografts in lethally irradiated mice. These results suggest that different NK cell subsets interact with each other, and affect their ability to reject BMC allografts in lethally irradiated mice. These results also suggest that these interactions may play a role in NK cell-mediated resistance to tumors and virally-infected cells. Studies focused on porcine-to-human xenografts addressed the role(s) of NK cells in transplant rejection. The host response to a xenograft is characterized by a large number of infiltrates by monocytes and NK cells suggesting an important role of the innate immunity in the process of its rejection. Mohanakumar et al. (Washington University School of Medicine, St Louis, USA) contributed information on the role of NK cells in modulating specific anti-porcine immune responses by secreting high levels of IFN-γ, through an IL-12 independent pathway. However, The IFN-γ response, however, was enhanced by IL-12 produced by macrophages upon activation by xenoantigens. The direct activation of NK cells by porcine endothelial cells and the species-specific IL-12 secretion by a unique macrophage activation pathway modulating the downstream specific immunity can provide a powerful effector mechanism in xenograft rejection. In a different model system, the abrogation of the ability of nude mice to reject xenografted metastatic carcinoma cell lines, by injecting anti-mouse NK cell antibodies, underscores the role of these cells in tumor rejection and suggests that by manipulating the innate immune system, it may not only be possible to discriminate cancer cells with and without metastatic potential, but also to modulate immunological parameters leading

to their selective killing (Kim et al., Buffalo Institute for Medical Research, Buffalo, USA).

The role(s) of NK cells in viral infection also generated great interest. The ability of human cytomegalovirus proteins to modulate NK cell lytic activity by affecting either cell surface expression and function of HLA-E, the ligand for the lectin-type NK receptors CD94/NKG2A and CD94/NKG2C/DAP12, was discussed by Ulbrecht et al. (Institut für Anthropologie und Humangenetik, Ludwig-Maximilians-Universität München, Munich, Germany). On the other hand, the role of NK cells as significant virus reservoirs in HIV-infected individuals may be due to their longer half-life as compared to the infected T-lymphocytes, as demonstrated by the use of GFP-tagged HIV-1 (Pavlakis et al., National Cancer Institute, Frederick, USA). Some of the previously reported defects in innate immunity after HIV infection may be the result of direct effects of viral infection of NK cell subsets.

5. Host-pathogen interactions

Contributions on innate immune responses to pathogens addressed topics from the microbes' colonization factors to cellular responses to infection, including the synthesis of anti-microbial peptides. The role of lectins as microbial colonization factors and the specific adaptive immune response was characterized (Mizrachi-Brauner et al., Ben Gurion University, Beer Sheva, Israel) by looking at the adherence of *Streptococcus pneumoniae* to mucosal epithelial cells. The binding of isolated bacterial surface proteins to epithelial cells was characterized by inhibition with sugars and human adult and children sera. The results indicate that children younger than 18 months fail to recognize these proteins, but after 24 months their serum inhibited 90% of the adhesion, suggesting that they may constitute good candidates for vaccine development. *Borrelia burgdorferi*, the causative agent of Lyme disease, expresses two adhesins which bind the collagen-associated proteoglycan decorin. The resistance to Lyme disease in decorin-deficient mice was examined by Brown et al. (Texas A&M University System Health Science Center, Houston, USA). Both tick and needle-inoculated mice consistently had fewer recoverable *Borrelia* from blood and joints, in addition to reduced arthritis incidence and severity. These data suggest a role for *Borrelia* adhesins in Lyme disease progression.

Reciprocally, novel information on the role of lectins in innate immune responses to infection by potential pathogens was contributed by Ofek et al. (Dept. Human Microbiology, Sackler Faculty of Medicine, Tel Aviv University, Israel). These investigators showed that macrophages recognize and kill, via the mannose receptor (MR), encapsulated serotypes of the lung pathogen, *Klebsiella pneumoniae*, which contain certain dimannose moieties. Lung macrophages may also encounter unencapsulated phase variants of *Klebsiella* which are not recognized by the MR, but are rather bound by the surfactant protein D (SP-D) that mediates their phagocytosis. In the studies presented at the meeting these investigators examined cytokine production by mononuclear phagocytes following their interaction with encapsulated *Klebsiella* via the MR, or with SP-D coated unencapsulated phase variants recognized by SP-D receptors. The results suggest that mononuclear phagocytes produce cytokines, upon stimulation with encapsulated or surfactant protein D-coated *K. pneumoniae*. Thus, macrophages might be aided by SP-D to elicit a protective response in the lung against unencapsulated phase variants, and concomitantly use their MRs to eliminate encapsulated dimannose-containing serotypes.

An interesting alternative mechanism for preventing pathogen adherence and colonization, although paying a rather heavy price to inherited stomach cancer, is illustrated by the Maori people of New Zealand, in which the evolutionary loss of function of the E-cadherin gene, the epithelial cell surface receptor for *Listeria monocytogenes*, a food-borne pathogen which causes meningitis and abortion in the immuno-compromised with a high case fatality rate (20–30%) world wide. The E-cadherin gene is also a tumour suppressor gene involved in normal cell-to-cell adhesion, and loss of function of this gene leads to uncontrolled cell detachment, tumor invasion and metastasis. The authors now provide evidence that a mutation giving rise to soluble E-cadherin blocks *L. monocytogenes* infection of the colon carcinoma cell line Caco2 by up to 66% and of gastric carcinoma cell line AGS by up to 87%

(Tatley et al., University of Otago, Dunedin, New Zealand).

The production of an antimicrobial peptide repertoire with membrane-damaging activity is an ancient innate immunity strategy. Defensins and cathelicidins are typical examples in mammalian phagocyte and epithelial defense. The latter are particularly variable from the structural standpoint, and Tomasinsig et al. (Natl. Lab. C.I.B., AREA Science Park, Trieste, Italy) contributed novel information on the expression of bovine cathelicidins, which, unlike the human homologue, appear to be restricted to myeloid-lymphoid organs and are upregulated in stimulated neutrophils, for example those infiltrating the lower airways in bovine pneumonia. Airway fluids from humans contain also contain antimicrobial peptides, among which calcitermin, a novel cationic peptide characterized by Cole et al. (Univ. of California, Los Angeles, USA) as a proteolytic cleavage product of calgranulin C, a zinc- and calcium-binding protein from neutrophils and monocytes, may play a role in the innate host defense of the airways. Direct testing of the in vivo protective activity of peptides present in respiratory secretions, such as the endogenous anionic peptide, was discussed by Brogden et al. (USDA, ARS, National Animal disease Center, Ames, USA). The effects of inflammation on expression of antimicrobial peptides in the human intestinal epithelium was assessed by Tjernström et al. (Department of Immunology, Umeå University, Umeå, Sweden) in specimens of patients suffering from ulcerative colitis, Crohn's disease from controls with no history of intestinal inflammation, and from colon adenocarcinoma cell lines. Inflammatory disease seems to alter the expression of α- and β-defensins in intestinal enterocytes, whereas results on the cell lines suggest that the state of cell differentiation is a factor in the expression of antimicrobial peptides.

The rapid activation of the antimicrobial peptide genes is one of major innate immune reactions in insects. In the silkworm, *Bombyx mori*, genes encoding cecropin A, cecropin B, lebocin (proline rich peptides with sugar chains), attacin (a glycine-rich peptide) and moricin are simultaneously activated upon injection with various different bacterial cell wall components. Using electrophoretic mobility shift assay (EMSA), two cis-elements were identified in the promoter of cecropin B gene (CecB), of which the CATTA motif appears to be the common LPS responsive cis-acting element in *B. mori* antibacterial peptide genes (Taniai, National Institute of Sericultural and Entomological Science, Tsukuba, Japan). A novel antifungal action mechanism was proposed by Kim et al. (Korea Advanced Institute of Science and Technology, Taejon, South Korea) by examining the action mechanism of tenecin 3, an antifungal protein from the coleopteran insect *Tenebrio molitor* by using a fluorescence dye leakage assay on the pathogenic fungus *Candida albicans*. Tenecin 3 did not act through the formation of membrane perturbing pores, but appears to be internalized into the cytoplasm of *C. albicans* exerting its antifungal activity through the induction of the vacuolar anion efflux.

Innate cellular response to pathogens also lead to cytokine and chemokine release. Interesting studies by Tabel et al. (University of Saskatchewan, Saskatoon, Canada) on experimental African trypanosomiasis indicate that upon phagocytosis of opsonized trypanosomes, macrophages produce cytokines which modulate the adaptive immune responses and that the degree of modulation depends on the number of trypanosomes phagocytosed. These results are consistent with the investigators' hypothesis that innate resistance to African trypanosomiasis is, at least partly, expressed at the level of the macrophage. On the other hand, the role of innate immune mechanisms for the resistance to urinary tract infections was underscored by results presented by Frendéus et al. (Lund University, Sweden) who used knockout mice lacking the IL-8 receptor to show increased susceptibility to acute experimental *E. coli* urinary tract infection, in parallel with TCR or RAG knockouts. Their results suggest that deficient IL-8 receptor expression may account for the increased susceptibility to pyelonephritis observed in some children. Susceptibility to fungal infections in newborns may be also due to 'less mature' innate immunity mechanisms. Activated large granular anti-fungal lymphocytes slow down the growth of the yeast and hyphal forms of *C. albicans* by reduction in the rate of DNA synthesis, and slowing progression of the fungal cell cycle (Mathews and Witek-Janusek, Loyola University of Chicago, Maywood, USA). As compared to adults, newborn infants have a heightened susceptibility to *C. albicans*, in part because the ability of both neonatal lymphocytes and PMNs to adhere and inhibit the growth of the

fungus is significantly less, and because infant mononuclear cells produce significantly less MIP-1α in response to *Candida*, suggesting a critical role of chemokines in antifungal immunity. The role of innate immunity in controlling viral infection was also addressed in detail. Among the presentations, the functional domains, regulation, and role of RNAse L (a 2′,5′-oligoadenylate-activated endoribonuclease that mediates antiviral actions of interferons) in mediating innate immunity, were characterized by Silverman et al. (Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, USA) by mutagenesis studies, RNAse L-null mice and transfected human cancer cells. The investigators propose that the antiviral activity of the 2-5A system (a series of 5′-phosphorylated, 2′,5′-linked oligoadenylates) may be due to the elimination of virus-infected cells through apoptosis.

6. Innate immunity, disease, and therapeutic interventions

Misdirected or uncontrolled innate immune responses may lead to abnormally enhanced inflammatory reactions and tissue damage, with potential to affect any organ or system. Tissue damage as a consequence of complement activation has been described in a wide variety of conditions such as experimental allergic neuritis, type II collagen induced arthritis, myasthenia gravis, hemolytic anemia, glomerulonephritis, immunecomplex-induced vasculitis and multiple sclerosis. Complement activation has also been implicated as a pathogenic factor in adult respiratory syndrome, Alzheimer's disease, stroke, heart attack, and burn injuries. Also as a consequence of complement activation by artificial surfaces in extracorporeal circuits, such as those encountered by patients undergoing dialysis, of after cardiopulmonary bypass, or by xenoantigens in transplantation and some gene therapies. Data presented by Sandor et al. (Department of Pathology, University of Wisconsin-Madison, USA) suggest that C3 also contributes to the lethality of LCMV-induced brain inflammation, and that complement inhibitors may rescue patients at the early acute phase of viral infection. Furthermore, as discussed by Köehl et al. (Institute of Medical Microbiology, MHH, Hannover, Germany), complement factors C3a and C5a play an important role in the pathogenesis of allergic asthma. When challenged by ovalbumin inhalation, an inbred guinea pig strain phenotypically unresponsive to C3a exhibited a decreased bronchoconstriction of approximately 30% as compared to the corresponding wild type strain. Furthermore, C5-deficient mice are susceptible to asthma, and the inhibitory effects C5 on cytokine (IL-12) production by macrophages may explain this observation.

The role of complement in chronic rejection of kidney transplants was addressed by Pratt (King's College, University of London, UK) by using a mouse model. Chronic allograft nephropathy results in gradual decline in renal function, and these investigators provide evidence that it may be caused by complement produced in the kidney, more specifically, in the proximal renal tubules, which are a significant source of complement. Thus, therapeutic approaches aimed at such a mechanism may assist in prolongation of transplant survival. Using a linear arrangement of membrane-binding units, termed Sequential Membrane Addressins, that in concert allow strong binding to cell membranes, these investigators targeted an active fragment of human complement receptor type 1 (CD35; a regulator of complement activation) to donor kidney tissue. The studies suggest that acute inhibition of innate immune mechanisms, such as the complement system, can produce significant benefits in transplantation.

Currently, promising candidates as inhibitors of complement activation for use in clinical applications are a recombinant form of complement receptor 1 known as soluble complement receptor 1 (sCR1), a humanized monoclonal anti-C5 antibody, two C5aR antagonists, and a small molecule known as 'compstatin' (Lambris, Department of Pathology & Laboratory Medicine, University of Pennsylvania, Philadelphia, USA) [5]. Compstatin, a disulfide-bonded cyclic peptide, inhibits complement activation by both the classical and alternative pathways. This molecule has been shown to be active in inhibiting complement activation in various models for extracorporeal circulation, hyperacute pig-to-human kidney rejection, and in vivo heparin-protamine complex induced primate complement activation.

Taking advantage of genetic determinants of disease resistance based on innate immunity mechanisms,

may constitute a strategy of wide application in the future. For example, the natural resistance associated macrophage protein (NRAMP), initially identified as a genetic determinant of resistance to intracellular parasites in the mouse, may find applications to selective breeding in agriculture as a new strategy to control zoonotic diseases. Although natural resistance to bacterial diseases is a result of multiple factors operating at the various levels of host-pathogen interactions, Adams et al. (Texas A&M University, College Station, TX, USA) has identified the bovine NRAMP1 as a major candidate gene involved in controlling intracellular bacterial pathogens. These investigators expect that the identification of additional candidate genes, microsatellite markers, and comparative gene mapping by will be conducive to a comprehensive view of the genetic basis of natural disease resistance and the development of practical applications.

7. Conclusions

Today's acknowledgment that the evolutionarily ancient mechanisms of innate immunity play major roles not only as the first barrier of defense against disease, but in rapidly and efficiently providing adaptive immunity with specific 'heads up' information about imminent infectious or neoplastic danger, has been buttressed by recent findings resulting from the implementation of state of the art approaches to address both self/non-self recognition and effector mechanisms. Further, the role of innate immune mechanisms in adverse inflammatory, autoimmune and allergic reactions, and the development of novel approaches for therapeutic interventions are currently topics of great interest, and subject to active investigation.

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