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Editors

COMPLEMENT

Second of Two Parts

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COMPLEMENT AT THE INTERFACE BETWEEN INNATE AND ADAPTIVE IMMUNITY

The formation of an antibody–antigen complex (immune complex) is the principal way of activating the classical pathway of the complement system. C1q, an integral part of the first component of complement (C1), triggers the activation process when it docks onto antibodies within these immune complexes. In this way, C1q acts to bridge the innate and adaptive immune systems.

Complement also has an important role in the induction of antibody responses.[62] This was shown first by Pepys, who demonstrated that the formation of antibodies against T-cell–dependent antigens was reduced in animals in which C3 had been depleted.[63,64] This result, which was considered heretical at the time, is consistent with our current knowledge of the way in which B cells become activated. A B cell begins to proliferate when antigen binds to its surface immunoglobulin molecules (antigen receptors). The activation of the B cell is modulated by coreceptors, which include receptors for complement components, especially complement receptor type 2.[65] When antigen meets a B cell in the presence of complement, the threshold for the activation of the B cell is lowered. Indeed, when antigen was coupled to C3dg molecules (C3dg is the main fragment of covalently bound C3, which is a ligand for complement receptor type 2), much less antigen (an amount smaller by a factor of up to 10,000) was required to evoke a given level of antibody than was the case for the native antigen.[66] This result supports the possibility of using complement as an adjuvant.

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How do newly encountered antigens activate and bind complement after entering the body? A subgroup of IgM antibodies, known as natural IgM antibodies, probably has a key role. Natural IgM antibodies are the products of immunoglobulin genes that have not undergone somatic mutation[67,68]; they can bind to many antigens, including microbial antigens and certain autoantigens. IgM enhances the production of antibodies by a mechanism that depends on C1q.[69] Mice lacking serum IgM antibodies have suboptimal responses of IgG antibody to low doses of antigen.[70] These findings suggest an important role for complement and natural antibodies in amplifying immune responses evoked by low doses of antigens.

COMPLEMENT AND INFLAMMATION

Complement that is activated at sites of tissue injury can cause damage through the deposition of the membrane-attack complex and cell-bound ligands, including C4b and C3b, that activate leukocytes bearing complement receptors. Complement can also amplify injury by means of the anaphylatoxins C5a and C3a, which cause the influx and activation of inflammatory cells. The two means by which complement is activated in tissues are through immune complexes, which activate the classical complement pathway, and through tissue ischemia and reperfusion, which expose phospholipids and mitochondrial proteins. These activate complement directly by binding C1q or mannose-binding lectin[71-73] or indirectly by binding natural antibodies[74] or C-reactive protein, which can activate the classical pathway by binding C1q.[75] Necrotic cells and tissues also lack the regulatory molecules that in normal tissues prevent the binding of complement.

COMPLEMENT AND NECROSIS

There is accumulating evidence that complement activation is an important contributor to the tissue necrosis that follows ischemia. Myocardial infarction and stroke are each associated with the activation of complement in the area of tissue infarction. In experimental models of these diseases, the inhibition of complement reduces the extent of tissue destruction.[76,77] The inhibition of complement in vivo is a relatively unexplored and possibly important way of preventing tissue necrosis.[78,79]

COMPLEMENT AND IMMUNE COMPLEXES

Complement may be friend or foe, depending on the circumstances. Under physiologic conditions, complement promotes the clearance of immune complexes, an important means of eliminating antibody-coated bacteria. If, however, immune complexes cannot be

eliminated, then complement becomes chronically activated and can incite inflammation. An example of this is an antibody response to an autoantigen, which cannot be cleared from the body. In Goodpasture's disease, the autoantigen is limited to particular organs, and only available for the binding of autoantibodies in the basement membranes of the glomeruli and lungs, the organs in which disease is expressed.

Chronic infections can perpetuate the formation of immune complexes, which in hepatitis C infection and bacterial endocarditis cause relentless activation and consumption of complement.

COMPLEMENT DEFICIENCY AND SYSTEMIC LUPUS ERYTHEMATOSUS

It has been widely accepted that the activation of complement by immune complexes is an important contributor to tissue injury in patients with systemic lupus erythematosus. However, the supporting evidence is largely circumstantial, amounting chiefly to an association between the activation of complement and the presence of injured tissue. Moreover, the fact that patients with hereditary deficiencies of complement proteins of the classical pathway are at increased risk for systemic lupus erythematosus[80] is hard to reconcile with the importance attributed to complement in this disease.

The strength of the association of complement deficiency with systemic lupus erythematosus itself and with the severity of the disease is inversely correlated with the position of the deficient protein in the activation sequence of the classical pathway. Thus, hereditary homozygous deficiencies of C1q, C1r and C1s, and C4 are each strongly associated with susceptibility to systemic lupus erythematosus, with respective prevalences of 93 percent, 57 percent (since deficiencies of C1r and C1s are usually inherited together), and 75 percent. By contrast, the prevalence of systemic lupus erythematosus among persons with C2 deficiency is about 10 percent.[80]

In the case of C3 deficiency, the clinical picture is rather different. It is characterized by recurrent pyogenic infections, membranoproliferative glomerulonephritis, and rashes (Fig. 4).

Could an ascertainment bias account for the association between deficiencies of certain complement components and systemic lupus erythematosus? There are three reasons why this explanation is unlikely. First, large population surveys have failed to identify any normal persons with a homozygous deficiency of a protein of the classical pathway.[7,81] Second, the concordance for disease among siblings in families in which more than one sibling has a homozygous deficiency of complement is 90 percent in the case of a C1q deficiency, 67 percent in the case of C1r and C1s deficiencies, and 80 percent in the case of a C4 deficiency. These values far exceed the degree of concordance for systemic lupus erythematosus among
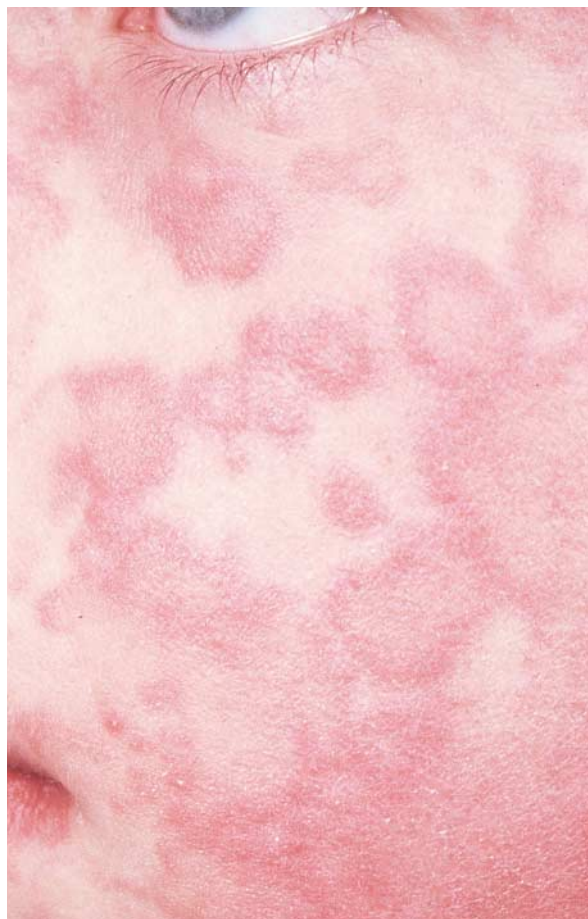

Figure 4. Facial Rash in a Patient with a Hereditary C3 Deficiency. This rash occurred each time the patient had a pyogenic infection of the respiratory tract, and in each instance, the rash lasted a few days.

monozygotic twins (24 percent).[82] Third, there is an association between systemic lupus erythematosus and hereditary angioedema. In patients with hereditary angioedema, excessive cleavage of C4 and C2 by C1s, caused by a heterozygous deficiency of C1 inhibitor, leads to an acquired deficiency of C4 and C2 that is sufficient to increase susceptibility to systemic lupus erythematosus.[80,83] The inference from these data describing the association of complement deficiency with systemic lupus erythematosus is that activation of the classical pathway of complement up to and including the cleavage of C4 has a powerful protective role against the development of systemic lupus erythematosus.

THE "WASTE-DISPOSAL" HYPOTHESIS

It is possible that complement has both inflammatory and antiinflammatory functions, the latter reflect-

ed by its role in clearing immune complexes from the circulation and removing them from tissues.[84] Complement also binds to cells that have undergone apoptosis[85-87] and helps to eliminate these cells from tissue.[88] If the complement system fails at this point, such waste material (consisting of partially degraded components of the cytoplasm and nucleus) could accumulate and evoke an autoimmune response. Figure 5 illustrates the three hypothesized steps to the development of systemic lupus erythematosus. The first step is the failure to clear autoantigens (i.e., defective waste disposal). This is the stage at which complement deficiency may have a pathogenic role. The second step is the uptake of autoantigen by immature dendritic cells in the presence of inflammatory cytokines, which causes these cells to mature into antigen-presenting cells, allowing the presentation of autoantigens to T cells. The third step is the provision by T cells of help to autoreactive B cells, which have taken up autoantigen by means of their immunoglobulin receptors. Such B cells mature into plasma cells that secrete autoantibodies. It is likely that in the majority of patients, systemic lupus erythematosus develops only in the presence of abnormalities in more than one of these steps. However, it is intriguing that in more than 90 percent of patients with a C1q deficiency, this defect alone appears to be sufficient to cause the expression of systemic lupus erythematosus.

One of the most striking features of the autoantibody response in systemic lupus erythematosus is that it is typically directed against many of the ubiquitous proteins and nucleic acids of multimolecular complexes. Apoptotic and necrotic cells may supply the autoantigens that drive the autoimmune response.

Experiments in mice provide support for this waste-disposal hypothesis. Mice lacking serum amyloid P component, which binds to extracellular chromatin and apoptotic cells,[89,90] have a defect in eliminating chromatin released by dead cells; in certain inbred strains with this deficiency, systemic lupus erythematosus develops in association with high titers of antibodies against both double-stranded DNA and chromatin.[91] A similar disorder occurs in mice lacking DNase 1, which digests extracellular DNA.[92] Mice lacking serum IgM also have a lupus-like syndrome, perhaps because natural autoreactive IgM promotes the clearance of inflammatory products and cellular debris.[93,94]

About one third of patients with systemic lupus erythematosus have high titers of autoantibodies against C1q. The presence of these autoantibodies is indicative of severe disease; they are strongly associated with severe consumptive hypocomplementemia and lupus nephritis.[95] The origin of these autoantibodies is unknown, but if C1q forms a molecular association with tissue debris, then it may itself become part of an autoantigenic complex.
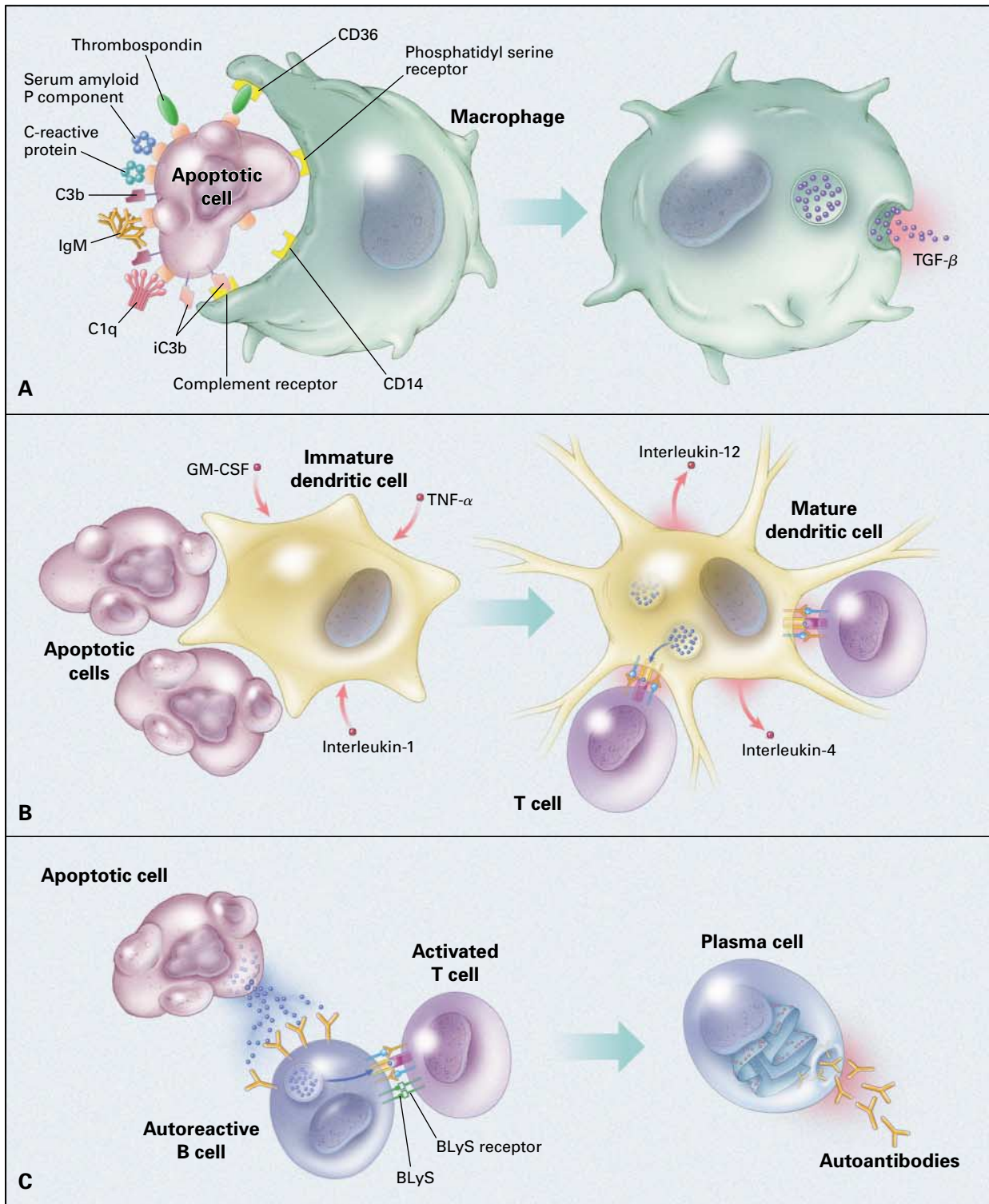
CONCLUSIONS

For many years research on complement was dominated by attempts to unravel the complex biochemistry. This work is largely complete, although important questions remain about the role of the mannose-binding lectin–associated serine proteases in the mannose-binding lectin pathway. There are hints of additional mechanisms of complement activation: ficolin, a molecule with lectin, fibrinogen, and collagen domains, may associate with these enzymes to activate complement.[96] We are still quite ignorant about much of the structural biology of the complement system, and this will be an important focus of research.

Studies of the immunobiology and genetics of complement are proliferating, encouraged by the availability of gene-targeted murine models of disease. Clinical studies have revealed the importance of inherited and acquired disorders of complement in many different human diseases. It has become apparent that the activation of complement in disease may be friend as well as foe.

The immune system has been studied predominant-

Figure 5 (facing page). The Waste-Disposal Hypothesis for Systemic Lupus Erythematosus.

In Panel A, a macrophage is shown engulfing an apoptotic cell. There are a variety of ligands on apoptotic cells and receptors on macrophages that make this process extremely efficient. The binding of C1q, C-reactive protein, and IgM to apoptotic cells may promote the activation of complement, leading to the clearance of apoptotic cells by ligation of complement receptors. The binding of serum amyloid P component masks autoantigen on the surface of apoptotic cells and promotes their safe disposal. Once the macrophage has engulfed the apoptotic cell, it secretes the antiinflammatory cytokine transforming growth factor β (TGF-β). As shown in Panel B, when there is an excess of apoptotic cells and the failure of one or more of the normal systems of receptor–ligand recognition for the uptake of apoptotic cells, immature dendritic cells may take up apoptotic cells. If this occurs in the presence of inflammatory cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), and interleukin-1, the dendritic cell may mature into an autoantigen-presenting cell. The dendritic cell is shown presenting autoantigens to a T cell in the presence of costimulatory molecules and cytokines. Panel C shows an autoreactive B cell that has taken up autoantigens from an apoptotic cell through its antibody receptors. The B cell is receiving help from an activated T cell, which is expressing costimulatory molecules and cytokines involved in the maturation of B cells, including an important member of the tumor necrosis family, B lymphocyte stimulator (BLyS), also referred to as zTNF-4. The autoreactive B cell divides and matures into a plasma cell that secretes autoantibodies. It is likely that in the majority of patients, systemic lupus erythematosus develops only in the presence of abnormalities in more than one of these steps.

A

Thrombospondin

Serum amyloid P component

C-reactive protein

C3b

IgM

C1q

iC3b

Complement receptor

CD36

Phosphatidyl serine receptor

Apoptotic cell

Macrophage

CD14

TGF-β

B

GM-CSF

Immature dendritic cell

TNF-α

Apoptotic cells

Interleukin-1

Interleukin-12

Mature dendritic cell

Interleukin-4

T cell

C

Apoptotic cell

Activated T cell

Plasma cell

Autoreactive B cell

BLyS receptor

BLyS

Autoantibodies

ly as an elaborate family of mechanisms for identifying and controlling infections. However, immunologists increasingly recognize that the immune system also patrols the internal milieu, identifying and removing damaged, neoplastic, or infected cells. There is a growing body of evidence that complement participates in this internal homeostasis and assists in the disposal of dead cells and immune complexes. A deficit in this function may underlie the association between complement deficiency and systemic lupus erythematosus.

Complement has been considered by many to be an arcane biochemical pathway participating in esoteric diseases. However, the involvement of complement in the necrosis that follows tissue ischemia suggests that future therapeutic approaches in stroke and myocardial infarction could focus on complement.

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