The role of immune complexes in disease

Report of a
WHO Scientific Group

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World Health Organization, Geneva 1977

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization

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* * *

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WHO SCIENTIFIC GROUP ON THE ROLE OF IMMUNE COMPLEXES IN DISEASE

Geneva, 22–28 September 1976

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THE ROLE OF IMMUNE COMPLEXES IN DISEASE

Report of a WHO Scientific Group

A WHO Scientific Group on the Role of Immune Complexes in Disease met in Geneva from 22 to 28 September 1976. The meeting was opened by Dr Ch'en Wen-chieh, Assistant Director-General, on behalf of the Director-General.

1. INTRODUCTION

An immune complex is produced when antigen interacts with antibody. The formation of immune complexes is a component of the normal immune response. It is only in uncommon situations that immune complexes trigger the sequence of injurious events that lead to disease. The essential feature in such diseases is the untoward activation, or inactivation, of the effector systems. Such mechanisms appear entirely physiological, yet it is their pathological consequences that are recognized as immune complex disease.

1.1 The composition of immune complexes

The nature of the complex formed depends upon several factors, including:

(1) The number and density of antigenic determinants within the molecule available for reaction with antibody. There may be multiple representation of a single determinant in a polymeric antigen or multiple different determinants. A molecule with a single antigenic determinant only can form complexes containing a single antibody molecule.

(2) The affinity of the interaction between antigen and the Fab portion of the antibody molecule. The affinity is usually in the range of 10^{12}–10^{4} litres/mole, although it is difficult to assign a lower limit for the affinity of biologically effective interactions. In test systems requiring relatively strong binding of antibody, certain low-affinity interactions may contribute to complex formation to a detectable extent only at reduced temperature. These so-called " cold " antibodies and the com-

plexes they form may nevertheless be important *in vivo* in causing disease.

(3) The ratio of antigen to antibody and the concentration of both.

(4) The modifications of the immune complexes occurring after their formation or deposition. During the course of dissociation and reassociation, the quantity and quality of antigen and antibody involved in complexes may change according to variations in the surrounding medium. This may affect the biological properties and the perpetuation of pathological effects.

The tendency for an immune complex to form and the properties of the complex formed depend on all these factors. Certain techniques not involving dilution or washing allow very weak interactions to be measured. From the biological standpoint, it is probable that immune complexes with the greatest pathogenetic potential are primarily those that can either activate plasma mediator systems, notably the complement system, or react with a number of cell types that have receptors for complexed immunoglobulin (Fc receptors) or bound complement (C3b and C3d receptors). However, other mechanisms also may be involved in complex-induced tissue injury.

Although immune complexes appear to be at the origin of several human diseases, the finding of complexes in any disease does not necessarily imply that they have a major pathogenetic role.

It is useful to distinguish between different circumstances in which the complexes are formed :

(1) Antibody reacting with antigen present as part of a membrane, either integral or passively attached. This is the type 2 allergic mechanism of tissue damage (*68*). The consequences of this type of reaction show characteristic features. Antibody that has bound to cell membrane components can be shed secondarily as a complex from the membrane into the fluid phase (*97*).

(2) Antibody reacting with soluble non-cell-bound antigen. This is the type 3 allergic mechanism of tissue damage (*68*). The consequences of this type of reaction vary with the location of complex formation.

Complexes can be formed : (i) when both antigen and antibody are blood-borne and secondarily localize in blood vessel walls and perivascular tissues (e.g., in serum sickness) ; (ii) when antigen locally released in the tissues reacts with blood-borne antibody (e.g., in onchocerciasis) ; and (iii) when both antigen and antibody are formed locally (e.g., late

granulomas around schistosome eggs). The two latter do not require any special localizing factors.

Most of the immune complexes in the circulating blood are rapidly cleared by the mononuclear phagocyte system, particularly Kupffer cells (*114*, *194*). This applies especially to large complexes and those that are complement-fixing. Complexes that are smaller or non-complement-fixing are cleared to some extent by the spleen, or they may become fixed to the renal glomeruli, blood vessel walls or choroid plexus (*48*, *145*). Elimination of antigen from the circulation by the mononuclear phagocyte system is inefficient when antibodies have low affinity for antigen (*8*). Therefore the nature and quantity of the complex detected in the blood is dependent on the dynamics of formation, clearance and tissue deposition of the immune complexes. It is likely that, in many instances, immune complexes that are capable of initiating injury never enter the circulation or are removed so rapidly that those circulating complexes detected by the various indicator systems are likely to be devoid of phlogistic activity. Thus, in such situations, the complexes should be regarded as reflecting the presence of injurious complexes that are deposited elsewhere.

2. MECHANISMS BY WHICH IMMUNE COMPLEXES CAN CAUSE TISSUE INJURY

2.1 The activation of plasma components

2.1.1 *Complement*

Immune complexes activate the complement system through both the classical and alternative pathways, although evidence in human beings indicates that the classical pathway is principally involved. IgG and IgM classes have this capacity, while those containing IgA, IgD and IgE do not. IgG1 and IgG3 are more effective in binding complement than IgG2 and IgG4 (*14*). After activation, C3 becomes bound at the site of immune complex deposition and is readily detected in tissues or on cells by fluorescent antibody techniques. The presence of bound C4 indicates involvement of activation by the classical pathway.

(a) Biological consequences of complement activation

As a result of activation of the complement system, several biological activities are generated that play a role in diseases of immune complex origin. These include :

(i) *C3 immune adherence*. This is a phenomenon in which leucocytes bind to C3b that is attached to membranes at the point of complement activation (*129*). Leucocytes, neutrophils and macrophages of all species studied, primate erythrocytes, and platelets of the rabbit and most other nonprimate mammals, except the ruminants, show this capacity. Membrane-bound C4b also induces the immune adherence of certain cells, although to a lesser extent than C3b. The immune adherence capacity of C3b is rapidly destroyed by C3b inactivator. The binding of leucocytes and nonprimate platelets to C3b bound to membranes is readily measured *in vitro* and may be of great importance in localizing these cells at sites of immunological reactions *in vivo*.

(ii) *Chemotaxis*. Chemotaxis of leucocytes, defined as the directional migration of cells provoked by a chemical stimulus, has been shown to be a property of activated complement components. The $\overline{C567}$ complex as well as C5a (or possibly a degradation product of C5a) are known to possess this capacity (*18*). C3a also has been found to be chemotactic for neutrophils. The effect is less marked than with C5a, and the possibility that the effect of C3a could be explained by a trace of contaminating C5a has not been entirely excluded.

(iii) *Exocytosis of neutrophil granules*. Immune adherence through the C3b receptor leads to exocytosis of neutrophil granules when phagocytosis cannot occur. It has been reported further that C5a can give rise to exocytosis of cytochalasin B-treated neutrophils, thus releasing their injurious content of enzymes and basic proteins to the exterior (*81*).

(iv) *Anaphylatoxin activity*. C3a and C5a can also stimulate mast cells to release their granules (see section 2.2.4).

(v) *Direct and indirect lysis of cells*. Cells become lysed by action of the terminal complement components when an immune complex is formed between an antigen present on a cell and a complement-activating antibody, or when an immune complex is brought into close apposition with the surface of certain cells (e.g., rabbit platelets, but not rabbit neutrophils). While the first condition is brought about by direct lysis, the second is a reaction of indirect or " bystander " lysis, the terminal components (C5–C9) becoming bound to the bystander cell (*185*).

(*b*) *The importance of the activation of complement in experimental immunopathological lesions*

The effect of complement activation has been observed in various immunopathological diseases. This has been shown usually by removal

of various complement components or by use of experimental animals genetically deficient in certain components. C3 and terminal components have been removed conveniently in a number of different animal species by treatment with a protein from cobra venom. The animals are rendered deficient in the components one or more days prior to the experiment. In rabbits so treated, the arteritic lesions, especially the accumulation of neutrophils, were prevented even though immune complexes were present. In Arthus reactions and nephrotoxic nephritis, neutrophils did not accumulate at the sites where immune complexes were located. Thus the attraction and/or binding of neutrophils required the biological properties of activated complement components.

However, the activation of the complement system does not appear to be always necessary for the development of lesions associated with immune complexes, particularly glomerulonephritis.

2.1.2 *The Hageman factor pathways : the kinin-forming, intrinsic clotting and fibrinolytic systems*

While early data suggested that immune complexes were capable of activating the Hageman factor pathways directly, subsequent studies have indicated that this is not the case (*49*). However, indirect activation may possibly take place when vascular basement membranes of mucopolysaccharides associated with collagen are exposed in the inflammatory process or when enzymes of cells such as neutrophils or macrophages are released.

2.2 Activation of cells by immune complexes

Immune complexes can activate a variety of cells by interacting with various surface receptors. Cellular activation can have several biological consequences. These reactions are summarized in Table 1 and discussed in this section.

2.2.1 *Platelets*

Human platelets bear receptors for the Fc portion of immunoglobulin, and they clump and release nucleotides and vasoactive amines in response to immune complexes or aggregated immunoglobulin. IgG1, 2, 3 and 4, but not the other classes of immunoglobulin, produce this response in platelets (*82*). Platelets also respond to thrombin, adenosine diphosphate, collagen, prostaglandin, platelet-activating factor of basophils and mast cells, adrenalin and many other agents by clumping, and in several of these instances, by releasing their contents of vasoactive amines and the

Table 1

Properties of human peripheral blood cells reacting with immune complexes

Cell type	Receptors for								Biological consequences of receptor activation		
	Cytophilic antibody [a]	Fc portion for IgG [b]	IgM	IgA	Complement components				Phagocytosis	Exocytosis [c]	Nonphagocytic cell killing
					C3b	C3d	C3a	C5a			
Platelets	0	1, 2, 3, 4	0	0	0 [d]				0	+	0
Neutrophils	0	1, 3 (2, 4) [e]	0	+ [e]	+		+	+	+	+	+
Eosinophils	0	1, 3			+		+	+	+	+	+
Basophils	IgE						+	+	0	+	
Monocytes	0 [d]	1, 3	0	0	+	0			+	+	+
B lymphocytes	0	+			+ [f]	+ [f]			0		
T lymphocytes	0	0 [f]	0 [f]		0 [f]	0			0		+ [f]
K cells	0	1, 2, 3, 4	0		+ [f]				0	0	+

+ = present; 0 = absent; blank = not known.

[a] Receptor capable of binding monomeric forms of immunoglobulin.
[b] Receptor reacts with indicated subclass of IgG.
[c] See text for materials released.
[d] Absent in man but present in other species.
[e] Evidence for receptor on neutrophils (controversial); binding of immunoglobulin classes (?).
[f] Present in a subpopulation of cells.

phospholipid procoagulant, platelet factor 3 (PF3). Basic proteins, somewhat analogous to the vasoactive proteins of neutrophils, are also contained within platelets. Considerable species differences exist regarding the receptors on platelets for aggregates of immunoglobulins and for C3 ; for example, the human platelet has receptors for the Fc portion of Ig and not C3b, while the rabbit platelet bears receptors for rabbit C3b but not Ig.

Data available concerning the role of platelets in the pathogenesis of immunopathological lesions are conflicting. In rabbit serum sickness, they appear to play a role in bringing about an increase in vascular permeability, allowing circulating immune complexes to become entrapped in the filtering membranes of the vessel walls (*48*). This presumably follows their clumping within the vascular lamina and the release of vasoactive amines. In Arthus lesions and nephrotoxic nephritis, their removal prior to challenge fails to alter the development of the disease. There are conflicting data on the role of platelets in the production of disseminated intravascular coagulation by bacterial endotoxin, but it is likely that in some circumstances they are required (*37*, *115*).

2.2.2 *Neutrophils*

Human neutrophils have receptors for the Fc region of IgG. Particles coated with IgG1 and IgG3 are phagocytosed. Attachment of erythrocytes coated with IgG antibody is followed by phagocytosis or contact-dependent lysis.

Contact between neutrophils and aggregated immunoglobulins or immune complexes leads to the release of granules from the neutrophils. This process liberates proteolytic enzymes and basic peptides into the medium. These increase vascular permeability, stimulate mast cells, and generate thromboplastin, which activates the intrinsic clotting system. In human systems, IgG1, 2, 3, 4, and IgA have been reported capable of such stimulation in aggregated form (*82*).

This process of exocytosis of neutrophil granules is probably responsible for much of the injury in acute immunologic nephritis in which neutrophils participate (there is also an important neutrophil-independent mechanism), acute immunologic synovitis, arthritis, and the vasculitis of the Arthus reaction. The release of superoxides, singlet oxygen, and peroxide may also play a role in the injurious process.

2.2.3 *Eosinophils*

In both guineapigs (*38*) and humans (*180*) eosinophils have receptors for the Fc region of IgG ; in the human cells these receptors are reported

to be selective for IgG1 and IgG3. Increased reactivity has been described in the cells from patients with eosinophilic states associated with cardiac damage.

Eosinophils have been shown to be cytotoxic to schistosomula (*39*) and *Nippostrongylus* (*144*) when these parasites are coated with antibody. The cytotoxicity involves close contact between effector and target cells. The energy metabolism, but not protein synthesis, of the effector cell must be maintained.

2.2.4 *Basophils and mast cells*

Although there is clear evidence that these two cell types are in many ways distinct they will be considered together in the present context.

Basophils have the capacity to bind IgE antibody (and possibly to a lesser extent some IgG anitbodies) cytophilically. Following the interaction of this fixed antibody with multivalent antigen the contents of the basophil granules are exocytosed. The granules contain heparin, histamine, slow-reacting substance of anaphylaxis (SRS-A), the eosinophil chemotactic factor of anaphylaxis (ECFA), and the platelet activating factor (PAF). Similar exocytosis can be produced by the reaction of the basophil with the complement fragments C3a and C5a. The receptors for these two closely related fragments are distinct, as demonstrated by the specificity of desensitization caused by each fragment.

Basophils and mast cells have assumed significance in the deposition of circulating immune complexes in acute experimental serum sickness of rabbits. An IgE-mediated anaphylactic trigger accompanies the deposition of circulating complexes and appears to be responsible for the increased vascular permeability that may be essential for the deposition of the circulating complexes in arteries and glomeruli (*48*).

2.2.5 *Mononuclear phagocytes*

Cells of this lineage arise in the bone marrow, circulate as monocytes, and carry out various differentiated functions as macrophages in the pulmonary alveoli, in the lining of the hepatic and splenic sinuses, and in the peritoneal cavity and other tissue spaces. Macrophages accumulate at sites of chronic inflammation. Mononuclear phagocytes in all these sites have the same receptors for immune complexes and complement components.

Human mononuclear phagocytes have receptors for the Fc region of IgG1 and IgG3; attachment of antibody-coated erythrocytes to these receptors is followed by phagocytosis or contact-dependent lysis (*84*). Incubation of mononuclear phagocytes with complexes of IgG antibody