Occasional survey

Immune complex disease

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Over the last decade it has become increasingly apparent that the deposition of immune complexes is a major feature of a wide range of human disease. Before discussing such disease processes it is necessary first to consider the immune complexes themselves and the factors which govern their composition, formation, and deposition in the tissues.

Complex composition

The complexes which result in disease usually have a molecular weight in excess of 500,000 and comprise both immunoglobulins and the antigens which stimulated their formation. The antigens responsible range from altered immunoglobulins, as in rheumatoid arthritis (Whaley and Carson Dick, 1969), to viruses (Györkey, Min, Sincovics, and Györkey, 1969; Dixon, Oldstone, and Toniatti, 1970; Onion, Crumpacker, and Gilliland, 1971), and complexes of virus and antibody have been detected both circulating and at the sites of tissue injury (Oldstone and Dixon, 1971).

Complexes which contain only immunoglobulins are 'mixed' (Cream, 1972) and may be $IgM \rightarrow IgG$ (the common rheumatoid factor), $IgG_1 \rightarrow IgG_3$, $IgG_3 \rightarrow IgG_1$, $IgA \rightarrow IgG \leftarrow IgM$, or $IgG \rightarrow IgA$ (The 'italicized' immunoglobulins are the ones whose production has been stimulated, whilst the arrows indicate against which other immunoglobulin they are directed).

Although these immunoglobulin complexes may precipitate on standing at 4°C., they are distinct from both the monoclonal cryoglobulins of myeloma and Waldenström's macroglobulinaemia and also from the cold aggregates which occur in cryofibrinogen-aemia and cold agglutinin disease.

Complex formation

Acute serum sickness is a systemic disease due to the deposition of relatively *small* complexes formed in the presence of an antigen excess. It represents hypersensitivity following a single antigenic challenge, becoming manifest about 7 to 14 days after the event.

The formation of soluble complexes after a single injection of a large amount of antigen such as horse serum is shown in Fig. 1.

FIG. 1 Formation of soluble complexes after a single injection of a large amount of antigen

Both acute and chronic serum sickness can, however, follow repeated antigenic challenge, complexes being formed either over a short period of time while circulating antigen combines with newly-formed antibody, or in low concentrations continuously over a long period (Dixon, Vasquez, Weigle, and Cochrane, 1958; Germuth, Senterfit, and Pollack, 1967b).

The Arthus reaction is the formation of focal erythema and oedema a few hours after the injection of soluble antigen into a *hyperimmunized* animal and is due to the formation of *large* immune complexes in the presence of a gross antibody excess.

Although serum sickness and the Arthus reaction are clinically very different, they are probably best considered as different parts of the spectrum of immune complex disease, separated by the size and composition of the complexes and the sites of deposition. These factors change with time, and features of both serum sickness and the Arthus reaction may be seen at different stages of a single disease process.

Complex solubility

The solubility of any complex varies with its composition. This is shown by the precipitin curve for ovalbumin and antiovalbumin (Fig. 2).

FIG. 2 Precipitin curve for a varying weight of ovalbumin (antigen) added to a fixed weight of antiovalbumin (antibody)

Complexes formed in antigen excess are relatively more soluble than those formed in the presence of an antibody excess, so that in gross *antibody* excess the complexes are rapidly precipitated and tend to be localized to the site of introduction, whereas in *antigen* excess soluble complexes are formed which may give rise to systemic disease.

Studies *in vitro* suggest that an equilibrium exists between soluble complexes, free antigen, insoluble complexes, and minute amounts of free antibody in the circulation (Germuth, Keleman, and Pollack, 1967a). This equilibrium can be expressed in terms of the overall formulae of the complexes.

This may be simplified to:

It has been suggested (Soothill and Steward, 1971) that immune complex disease occurs particularly when poor affinity antibody is produced in response to antigenic challenge. The result of this is first that an incomplete immune elimination of antigen occurs and secondly that any small amounts of high affinity antibody which might also be produced would therefore be formed in a continuing antigen excess and likely to form highly soluble complexes.

Complex deposition

Normally any relatively insoluble complex formed in the circulation is rapidly removed by the phagocytic

cells of the reticulo-endothelial system. In immune complex disease, however, complexes become deposited in some tissues where they may remain for many weeks sequestered from the phagocytic cells of the blood.

Apart from the synovial membrane of joints, the main sites of complex deposition are the walls of the blood vessels, particularly in the kidney and skin.

It is possible that, during diffusion through vascular walls, soluble complexes undergo re-equilibration to less soluble complexes (see above) because of the more rapid migration of free antigen through the vessel wall. This removal of free antigen, which is in equilibrium with each of the above formulae, will cause a 'shift to the right' in the overall equilibrium.

In the renal glomerulus complexes may be deposited on the epithelial side of the basement membrane, in skin venules they may be deposited in the capillary loops of the dermal papillae, especially around the basement membranes, and in arteries there may be deposition in the elastic lamina.

Factors affecting complex deposition are numerous and affect the site of deposition, both as regards the organ involved and also the site of complex deposition with the organ.

(1) Hydrostatic Pressure

Differences in hydrostatic pressure are most important in the deposition of complexes in the kidneys and skin and, if such differences are abolished, as in the case of the kidney, either by renal artery stenosis or by hydronephrosis, the affected kidney is protected from the deposition of immune complexes (Germuth and others, 1967a).

Acute glomerulonephritis is found mainly in glomeruli situated close to the medulla (and consequently also to the primary blood supply), and this may be due to a higher intraglomerular hydrostatic pressure in these glomeruli than in those in the outer parts of the cortex and further from the primary blood supply (Germuth and others, 1967a).

(2) Vascular permeability

Increased vascular permeability facilitates the deposition of complexes in vessel walls and the local release of vasoactive amines has been suggested as part of a mechanism resulting in complex deposition (Cochrane, 1971).

(3) Complex concentration

A low concentration of soluble complexes present for a long time will cause glomerulonephritis, whereas arterial lesions require a higher concentration of complexes for their development (Germuth and others, 1967a).

(4) Complex composition

This is probably one of the main factors influencing the exact site of complex deposition within the tissues. It would be expected that highly soluble complexes with formulae of the order of Ag_2Ab might penetrate to greater distances within blood vessel walls than those with formulae of the order Ag_3Ab_2 . Indeed, the Arthus reaction is primarily associated with damage to venular endothelium while fibrinoid necrosis of arterial elastic lamina is associated with serum sickness.

(5) Time

Complex composition changes with time, but the distribution of arterial lesions also changes with the duration of the antigenic stimulation (Heptinstall and Germuth, 1957). Whilst coronary and pulmonary arteries may be involved in both acute and chronic serum sickness, renal vasculature is hardly ever affected in acute serum sickness, whereas renal arcuate and interlobar arteries may show lesions in chronic serum sickness.

Factors affecting the deposition of complexes within the joints are less clearly understood, but probably high local concentrations of complex and poor lymphatic drainage (and hence complex clearance) are two of the main ones.

EVENTS FOLLOWING THE DEPOSITION OF COMPLEX

When antibodies become fixed to antigens, the Fc portions of their heavy chains become 'activated' and may then either attract nonimmune (uncommitted) circulating lymphocytes (probably of the 'B' type) or activate the complement cascade (Hobbs, 1971). Neither of these mechanisms are clinically important while the complex is circulating, but both can lead to tissue damage when the complex has been deposited.

The main events following the deposition of complex are outlined in Fig. 3, and the cellular aspects of this sequence of events are also shown in Diagram 1.

Complement is usually detectable in deposited complexes which are associated with the more usual polymorphonuclear leucocyte infiltration, whereas it is unusual to detect complement in complexes associated with the less usual infiltration of non-immune lymphocytes.

In terms of tissue damage the critical events are:

- (1) Attraction of nonimmune lymphocytes and the production of cytotoxic factors (Hobbs, 1971).
- (2) Activation of complement.
- (3) Attraction of polymorphonuclear leucocytes and the subsequent release of proteolytic lysosomal enzymes (Ward, Cochrane, and Müller-Eberhard, 1965).
- (4) Deposition of fibrin—this probably occurs over a fairly short period of time and makes the lesions irre-

versible. It is fibrin deposition which accounts for the 'wire-loop' lesions of systemic lupus erythematosus (SLE).

- (5) Vasoactive amine release by platelets.

FIG. 3 Summary of events after the deposition of complex in tissues (modified from Roitt, 1971)

DIAGRAM 1 Sequence of events following the deposition of complex, showing the attraction of polymorphs when complement is activated and the attraction of lymphocytes when complement is not activated

HISTOLOGICAL FEATURES ASSOCIATED WITH THE DEPOSITION OF IMMUNE COMPLEXES

The above sequence of events may lead to a number of histological pictures (Humphrey and White, 1970).

The Arthus reaction in skin is characterized by a massive neutrophil infiltration, gross oedema, local segmental necrosis of venules and arterioles, and platelet-leucocyte thrombi after a few hours, with later neutrophil degeneration and the accumulation of lymphocytes, macrocytes, and eosinophils.

In the kidney in acute serum sickness and some forms of post-streptococcal nephritis, there is swelling and proliferation of endothelial cells, swelling of stalk cells, and the patchy appearance of polymorphonuclear leucocytes. There is also scattered focal thickening and fraying of the glomerular basement membrane, while the epithelial cells remain fairly normal.

In chronic nephritis, complex deposition on the epithelial side of the basement membrane results in a lumpy thickening of the basement membrane with marked disorganization of the foot processes.

Another course which may be taken is seen in experimental rabbits with *extremely* high antigenic challenge where *granulomata* are seen in addition to the necrotizing arteritis and the necrotizing and proliferative glomerulonephritis of protracted serum sickness (Germuth and Pollack, 1967). They occur in pulmonary veins and the tissue surrounding them, spleen, lymph nodes (particularly in the Malpighian follicle of the mantle zone), and sometimes in hepatic portal spaces. These granulomata are discrete collections of epithelioid and giant cells and are the histological reflections of the removal and sequestration of large insoluble complexes by reticulo-endothelial cells (Germuth and Pollack, 1958).

Clinical features of immune complex disease

The clinical features of immune complex disease are very numerous. The main ones are set out in Table I.

Table I *Main clinical features of immune complex disease*

Fever	
Generalized lymphadenopathy	
Hypertension	
Albuminuria	} — Glomerulonephritis
Oliguria	
Haematuria	
Oedema	
Maculopapular rash	} — Cutaneous vasculitis
Leg ulcers	
Purpura	
Raynaud's phenomenon	
Arthritis	
Hepatitis	
Myositis and myocarditis	
Generalized urticaria	

The nodular cutaneous vasculitis (Fig. 4) associated with *mixed* cryoglobulinaemias is distinct from the flat, often ulcerated skin lesions associated with the monoclonal cryoglobulinaemias.

Obviously not all the features listed in Table I will be present all the time and the actual expression of disease will depend on the antigen, the dose received, the route of administration, and the length of time after the challenge.

DISEASES ASSOCIATED WITH THE DEPOSITION OF IMMUNE COMPLEXES

It is convenient to divide immune complex diseases into generalized and localized disorders, these being associated mainly with the presence of small and large complexes respectively.

Examples of generalized diseases are rheumatoid arthritis (Whaley and Dick, 1969), systemic lupus

FIG. 4 *Nodular cutaneous vasculitis on the forearm of a patient with immune complex disease*

erythematosus (SLE) (Christian, 1969), some forms of post-streptococcal nephritis (Markowitz and Lange, 1964; Dixon, Wilson, and Marquardt, 1971), malarial nephritis, hypersensitivities to drugs such as penicillin (Copeman, 1972), and probably various virus infections. SLE may represent a reaction to myxovirus nucleic acid with cross-reaction against self nucleic acid (Györkey and others, 1969).

Localized diseases include farmer's lung (an inhalational disease characterized by the formation of intrapulmonary Arthus-type reactions in patients sensitized to thermophilic *Actinomyces* from mouldy hay), pigeon fancier's lung (a hypersensitivity to inhaled protein antigen from dried bird faeces), erythema nodosum, insect bites, some complications of viral diseases such as post measles encephalitis and rubella arthritis, and possibly some complications of bacterial diseases such as gonococcal arthritis.

This list, which is summarized in Table II, indicates the wide spectrum of diseases in which the deposition of immune complexes has been seen. As more becomes known about the pathogenesis of disease, undoubtedly the list will grow.

Table II *Examples of immune complex diseases*

Generalized	Rheumatoid arthritis
	Systemic lupus erythematosus
	Post-streptococcal nephritis
	Malarial nephritis
	Penicillin hypersensitivity
Localized	Farmer's lung
	Pigeon fancier's lung
	Erythema nodosum
	Insect bites
	Post measles encephalitis
	Rubella arthritis
	Gonococcal arthritis

Diagnosis of immune complex disease

The two main lines of investigation are examination of the serum for circulating complexes and complement studies and of tissue biopsies for deposited complexes.

(1) Serum

It is possible to look for the presence of immune complexes either by their slow precipitation at 4°C., which may take up to 3 or 4 days (Cream, 1972), or by the more modern technique of ultracentrifugation. In the presence of a gross excess of complexes, a 'shoulder' appears between the 7S and 19S peaks (Diagram 2).

Serum complement studies may also be performed. The two investigations likely to be most helpful are C₃ (β_{1c}) levels, since hypocomplementaemia is often a feature of serum sickness, especially in SLE (Schur and Sandson, 1968; Kohler and Ten Bensel, 1969) and the presence or absence of complement

DIAGRAM 2 *Ultracentrifugation patterns of normal serum (A) and serum with a gross excess of complexes (B), showing the positions of the 4.5, 7, and 19S peaks and the 'shoulder' due to complexes*

conversion products, which may act as a pointer to the activity or otherwise of the disease process (Schur and Austen, 1968). Both these investigations are best performed by crossed immunoelectrophoresis. Fig. 5 shows both the reduction of C₃ in a patient with SLE compared with a normal subject and also the presence of conversion products (Versey, Hobbs, and Holt, 1973).

(2) Tissue biopsy

By immunofluorescent staining techniques it is possible to detect antibody, complement, fibrinogen, and antigen in tissue biopsies. All 4 may be demonstrable together in tissue such as skin from patients with immune complex disease (see Fig. 6, overleaf).

Treatment of immune complex diseases

Although this is somewhat empirical at the present time, as the processes involved in immune complex diseases become better understood a more rational approach will be possible.

FIG. 5 *Two-way immunoelectrophoresis of serum from a normal subject (A) and a patient with SLE (B), showing the positions of the origin (O), albumin (alb), transferrin (tf), C₃ component of complement, and C₃ conversion products (C₃i).*

FIG. 6 Sections of skin blood vessels from a patient with immune complex disease, all taken at the same time and stained by immunofluorescence for IgG (A), complement (B), fibrinogen (C), and streptococcal antigens (D)

Therapy available at the present time includes:

- (1) Steroids, which stabilize lysosome membranes, thus preventing the release of proteolytic enzymes, reduce the number of circulating co-optable lymphocytes, and also affect platelet and complement function.
- (2) Antihistamines, which can block the actions of some of the vasoactive amines which may be released.
- (3) Heparin, which prevents fibrin deposition, although if used it must be used early in the

disease process, since it is not effective once fibrin has begun to be laid.

Other drugs not in use at present may also be used in the future, possibly to block phagocytosis, to 'dissolve' fibrin which has already been deposited, to block the activation of complement, or to alter the balance of antibody and antigen (Soothill and Steward, 1971). Antibody-antigen ratios might be changed by giving immunosuppressive drugs, by infusing antibody, or by giving further antigen, possibly with adjuvant.

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