Autoimmune diseases fall into two broad categories: tissue-specific, and tissue non-specific or systemic disease, in which a range of tissues are involved. In tissue-specific disorders, the pathology of the disease reflects the specificity of the auto-antibody and myasthenia gravis is a good example with antibodies against the nicotinic acetylcholine receptor being present in circulation. Myasthenia gravis [4, 5] and other tissue-specific autoimmune disorders [2, 3] will be discussed later in the colloquium.

Systemic autoimmune disease covers disorders in which several tissues may be affected and a range of antibody specificities are found [4, 5]. The disorders (and their symptoms) include rheumatoid arthritis (inflamed joints), polymyositis (inflammation of striated muscle), scleroderma (fibrous tissue deposits in skin and internal organs), Sjogren's syndrome (dry mouth, dry eyes, inflamed joints) and systemic lupus erythematosus (vasculitis, joint pains, rash and nephritis). Antibody specificities are not necessarily exclusively associated with a particular condition, for example anti-DNA antibodies are found in Sjogren's syndrome and in systemic lupus erythematosus (SLE). Many of the antibody specificities which are found are for intracellular antigens [4, 5] and anti-ubiquitin antibodies have been described [6]. Other intracellular antibody specificities include anti-histone antibodies, anti-ribonuclear proteins and anti-cardiolipin antibodies although cross-reactivity with circulating cell-associated [7] and plasma protein antigens [8] have been observed. Many of the subcellular antigens have been cloned [3, 22, 23] and their role in the pathogenesis of the disease is not yet clear.

Many of the subcellular antigens have been cloned and their role in the pathogenesis of the disease is not yet clear.

The symptoms of SLE, considered as the prototypical immune complex disease [11] can be understood not so much in terms of the specificities of the antibodies which are found but in the forma-
tion of immune complexes between antibody and antigen. Many of the antibody specificities which are found in systemic autoimmune disease are also encountered as natural antibodies; that is, they cover the same specificity range as low-affinity antibodies, usually IgM, which are found in serum of normal individuals [12]. It may be postulated that, since cell death is occurring constantly in all individuals, these antibodies are involved in removal of cell debris [4]. The conversion of the low level of these antibodies in normal individuals into immune complex disease must have some underlying key event. The nature of this trigger is not known but certain drugs will induce an autoimmune condition which resembles SLE [13]. The drugs associated with immune complex disease as an adverse side-effect include drugs given for hypertension (hydralazine), cardiac arrhythmia (procainamide), epilepsy (phenytoin) and rheumatoid arthritis (penicillamine) (Fig. 1) and the toxicity is unlikely to be caused as a result of the pharmacological action of these drugs. In addition, human exposure to environmental agents (hydralazine) [14] and exposure of monkeys to canavanine in the diet [15] has also been associated with development of SLE-like symptoms. Although the pattern of antibody specificities found in drug-induced SLE and the clinical picture of the resulting immune complex disease may differ in the idio-pathic and drug-induced immune complex disorders, the spontaneous immune complex diseases themselves can have overlapping antibody specificity patterns and clinical pathologies [4, 11].

**Fig. 1**

**Drugs and metabolites involved in drug-induced SLE**

\[
\begin{align*}
\text{Hydralazine} & : \text{NH}_2 \quad \text{NH} \\
\text{Hydroxylamine} & : \text{O} \\
\text{Procainamide} & : \text{H}_3 \text{C}-\text{C}-\text{C}=\text{OH} \\
\text{Penicillamine} & : \text{H}_2 \text{C}=\text{C}=\text{C}=\text{OH} \\
\end{align*}
\]

Immune complex diseases are considered to be disorders of immune complex handling [16-19] in which the ability of an individual to deal successfully with an immune complex load becomes overwhelmed (e.g. as a result of infection) and results in immune complex deposition at inappropriate tissue sites. While this approach does not provide an answer to the triggering event in SLE, for example, it allows the cyclic nature of the disorder to be understood [4]. Drug-induced immune complex disease develops after long-term therapy, for example with the anti-hypertensive drug hydralazine, a mono-substituted hydrazine, at least 100 g of the drug must be ingested and less than 15% of individuals suffer the adverse reaction [20]. This time scale and pattern of onset of hydralazine-induced SLE suggests that it is unlikely that the drug provides the sole trigger for an individual to go on to develop drug-induced immune complex disease.

After two years’ therapy with the arrhythmic drug procainamide, which is an arylamine, 100% of individuals develop anti-nuclear antibodies though not all patients go on to develop an SLE-like condition. A particular profile of antibody specificities has been described in procainamide-induced SLE [21] which may point to a role of the drug in antibody production. The time-scale and frequency of those who develop an adverse side-effect to these drugs, suggests that drug-induced SLE, like spontaneous immune complex disease, is a disorder in which a range of predisposing factors are important and are required to be present simultaneously in an individual for development of the disease [22]. Drug-induced SLE provides a human model for a multifactorial disorder in which one environmental factor, the drug, can be unambiguously identified.

The approach which has been taken in the present study is to consider the effect of drugs which induce SLE on the ability of an individual to cope with immune complexes. The drugs which will be discussed specifically in this article are hydralazine, procainamide and penicillamine which are themselves nucleophiles or which are metabolized to nucleophilic compounds.

**Immune complexes and complement activation**

Immune complexes are formed in all individuals, but in healthy individuals, immune complexes are removed through the reticuloendothelial system and the removal of immune complexes is promoted through activation of the complement cascade [16-19]. The classical pathway of complement
appears to play a crucial role in the removal of immune complexes since individuals who are deficient in the classical pathway complement components (C1, C4 or C2) are at increased risk of developing SLE [23].

Activation of the complement system [24] promotes removal of immune complexes through three mechanisms, all of which involve activation and binding of the central component, C3. Activation of the classical pathway of complement by immune complexes leads to the covalent binding of C4 (as C4b) on the surface of the immune complex. This covalently bound C4b leads in turn to activation of the alternative pathway and thus to amplifi-

**Fig. 2**

**Activation and covalent binding reactions of C3 and C4**

The C1 component of complement interacts through the Clq subcomponent with the Fc region of antibody molecules in immune complexes with antigen (shown by the hatched box). This in turn activates the C1s subcomponent, a protease, which cleaves C4 to C4a, an anaphylatoxin of 8 kDa, and the rest of the molecule (192 kDa), 'nascent' C4b, which has the short-lived exposed and activated thiol-ester site. C1s also cleaves C2 to C2a and C2b. The C2a fragment together with C4b forms the C3 convertase of the classical pathway. C3, like C4, is cleaved to form C3a and 'nascent C3b'. The C3 convertase of the alternative pathway, C3bBb, also cleaves C3 in the same way and it is C3bBb which is responsible for amplification of C3 activation [24]. Nascent C3b or C4b can react with oxygen (route A) or nitrogen (route B) groups on the surface of immune complexes (hatched box). It is through these reactions that C3b and C4b become bound to the surface immune complexes. Nascent C3b or C4b can also react with soluble oxygen nucleophiles, including water (route C). Soluble nitrogen nucleophiles, including hydralazine (route D), can also bind covalently to the thiol-ester site. The hydroxylamine metabolite of procainamide is likely to react through routes C and D. Route E shows the proposed reaction of α-amino, β-thiol compounds with the exposed thiol-ester [41]. Penicillamine is such a compound in which R is CH3.
cation of covalent binding of C3 (as C3b) on the surface of the immune complex (Fig. 2).

The covalent binding mechanism of C4 and C3 on the surface of immune complexes (Fig. 2, routes A and B) involves nucleophilic attack of a thiol-ester group in these proteins by oxygen or nitrogen nucleophilic groups on the surface of the immune complex [24]. The thiol-ester group in C3 and C4 is buried in the native protein but becomes exposed and reacts with adjacent nucleophiles when C3 or C4 is activated by proteolysis at a distant site [24, 25]. Nucleophiles in solution can compete for the binding reaction of activated C3 and C4 to immune complex surfaces and fluid-phase nucleophiles themselves become covalently bound to C3b or C4b [26]. When C3 or C4 is inhibited from binding to the surface of an immune complex in this way it cannot participate further in the complement activation sequence on the immune complex surface. Since the half-life of activated C3 or C4 is very short (less than 1 ms), any fluid-phase nucleophiles must be present during complement activation in order to compete with nucleophilic groups on the immune complex surface for covalent binding of C3b [26] or C4b. Water competes effectively in this binding reaction (Fig. 2, route C) and only approx. 10% of C3b or C4b which is activated normally becomes covalently bound to the complement-activating surface. The degree of inhibition of the covalent binding of C3 [27] and C4 [28] to complement-activating surfaces is directly dependent on the nucleophilicity of the fluid-phase nucleophile and in the presence of additional competing nucleophiles, the amount of C3b [26] or C4b [29] which is bound to a complement-activating surface, decreases.

C3b bound on the immune complex surface contributes to removal of immune complexes through: (1) Opsonisation of the complex: that is through promoting the interaction between phagocytic cells and immune complexes. This occurs both directly through receptors (CR1 and CR3) on the surface of phagocytes and also indirectly through transport of immune complexes, bound to CR1 on erythrocytes [17, 18, 30], to tissue phagocytes for ingestion; (2) Controlling immune complex size. Covalent binding of C3b causes solubilization of pre-formed immune complexes [31]. It is likely that C3b deposition on immune complexes as a result of activation of the classical pathway provides a mechanism in vivo for limiting the size of the immune complexes which are formed. This is referred to as inhibition of immune precipitation [18]; and (3) Attracting phagocytes to the vicinity of immune complexes. C3b covalently bound to immune complexes forms an essential component of the enzymes responsible for C5 activation. These enzymes, called C5 convertases, lead to proteolytic activation of the C5 component of complement and the activation fragment C5a acts as an anaphylatoxin and as a chemotactic factor. C5a is one of the most potent chemotactic factors known for neutrophils [32].

Therefore, inhibition of the covalent binding of C3b to immune complexes, either directly or indirectly through inhibition of the covalent binding of C4b, has adverse effects on immune complex handling leading to larger immune complexes persisting for longer periods in circulation. These effects are likely to increase tissue deposition of immune complexes, particularly in small blood vessels. The deposition of immune complexes in skin and in kidney glomeruli has been suggested as being due to the deposition of soluble complexes on surfaces with a large filtration area [4]. Once immune complexes become deposited at these tissue sites they can act as a focus for further immune complex deposition and also promote complement activation, cellular infiltration and tissue damage [33].

**Drugs**

Hydralazine is a nitrogen nucleophile and has been shown to inhibit the covalent binding reaction of C4 to complement activating surfaces [29]. Inhibition of the covalent binding of C4 by hydralazine is associated with the covalent binding of the drug to the polypeptide chain of C4b which contains the active site thiol-ester of C4. The drug only becomes bound to C4b if it is present during activation of C4 [34]. From these results it is likely that hydralazine binds to the active carbonyl in C4 (Fig. 2, route D). Hydralazine is metabolized by N-acetylation of the hydrazine group [35]. This reaction is catalysed by N-acetyltransferase which is expressed polymorphically in the human population [36]. Although the ‘fast’ acetylator phenotype is more common, hydralazine-induced SLE occurs almost exclusively amongst ‘slow’ acetylators [37]. These results are compatible with the drug rather than its acetylated metabolite being the toxic agent. In accordance with this finding, the major acetylated metabolite of hydralazine, methyl-triozolophthalazine could not be shown to inhibit the covalent binding of C4b to an activating surface [29].

In addition to differences amongst individuals in their ability to metabolize hydralazine, C4 as the target molecule is also polymorphic. C4 is encoded
at two loci, C4A and C4B within the major histocompatibility complex (C4A and C4B are used to refer to the protein products of different C4 genes; C4a and C4b are different proteolytic activation products of C4 produced). At each of these loci there are multiple alleles, including null or non-functional alleles [38]. The gene products of the C4A and C4B loci have been found to have limited protein sequence differences [38]. These C4A- and C4B-type-specific amino acid differences are clustered in a group of six amino acids. There is an important difference in the reactivity of the products of the C4A and C4B genes in that the exposed thiol-ester of the C4A gene product is much more reactive with nitrogen [39, 40] and also sulphhydril nucleophiles [41] than is the exposed thiol-ester of the C4B gene product. This suggests that the specificity-conferring region of C4, which is on the C-terminal region some 120 amino acids distant from the thiol-ester site in C4, is adjacent in three-dimensional structure to the thiol-ester site. The conformation of the protein changes on activation and it is not possible to say whether the juxtaposition of the thiol-ester and specificity-conferring regions of C4 changes on activation to generate the environment determining the binding specificity of the thiol-ester site carbonyl group. The aspartate and cysteine residues in C4A (Fig. 3) have been suggested to be responsible for the increased binding of C4A to nitrogen and sulphhydril nucleophiles, respectively.

Hydralazine, like other nitrogen nucleophiles is more reactive with C4A than with C4B [34]. The amount of hydralazine (500 μM) which is required to inhibit the covalent binding of C4A by 50% is four times less than that required in the case of C4B. In plasma, it has been observed that the amount of C4A and C4B found in plasma can be correlated with the number of null alleles which are present [42] and in individuals with null alleles it may be that inhibition of the reduced amount of C4 which is present is important as a pre-disposing factor in hydralazine-induced SLE. The importance of C4 type in determining susceptibility to SLE has been demonstrated by the increased frequency with which null alleles for C4A are found [43, 44]. C4 null alleles are also found with increased frequency compared with a control population in hydralazine-induced SLE [45]. However, in this latter study, there was no difference found between the increased incidence of C4A null or C4B null alleles in the disease populations.

Procainamide is an arylamine which is not strongly nucleophilic and is a poor inhibitor of the covalent binding of both C4 and C3 [46]. However, procainamide is metabolized to a nucleophilic hydroxylamine form [47, 48] and the hydroxylamine form of procainamide has been shown to be much more effective as an inhibitor of the covalent binding reaction of C4 and C3 than is the native drug [46]. Although 50% inhibition of C4 and C3 binding occurs at 1–2 mM of the hydroxylamine form, inhibition could be detected at 10-fold lower concentrations. The N-oxidation of procainamide (and other arylamines) has been shown to occur in liver preparations [49], but additionally, generation of the hydroxylamine metabolite of procainamide has been shown to occur in response to ingestion of particles by polymorphonuclear leucocytes and mononuclear phagocytes [47, 48]. This is particularly important in view of the interaction between the complement system and phagocytes in immune complex clearance. Procainamide-induced SLE requires long-term exposure to the drug [50] and the quantitative importance of the hydroxylamine metabolite needs to be assessed under these dosage conditions.

As well as N-oxidation, the amino group of procainamide is metabolized by N-acetylation and there is a more rapid onset of procainamide-induced SLE amongst slow acetylators [51]. It has been suggested that in slow acetylators more procainamide is available for other metabolic routes such as oxidation. In humans, N-acetyltransferase activity is found in a wide range of tissues, including liver, tonsil lymphocytes and in the monocytic cell
line U937 [52]. However, compared with liver, extra-hepatic sources are likely to contribute little to the acetylation of procainamide. This further emphasizes the likely importance of local oxidation in phagocytes. It has been demonstrated that stimulated mononuclear phagocytes secrete all of the components of the alternative pathway of complement such that particles become coated with C3b which then stimulates their uptake by CR3, a cell surface receptor for the C3b fragment iC3b [53]. Therefore, the role of oxidized metabolites of procainamide in the microenvironment of phagocytes in controlling the deposition of C3b on immune complexes and their subsequent ingestion by phagocytic cells may be important.

The anti-rheumatic drug, penicillamine-2,2-dimethylcysteine, is an α-amino, β-thiol compound. Penicillamine is associated with a wide range of adverse symptoms which may occur in up to 50% of individuals [54]. Adverse reactions which develop after long-term therapy may rarely include myasthenia gravis [4], but symptoms suggestive of immune complex disease are often reported [55]. These may resemble SLE but problems of proteinuria are common and glomerulonephritis can occur. The effect of penicillamine on the covalent binding reaction of C4 has shown clearly that penicillamine inhibits the covalent binding of C4b if it is present during activation of C4 [41]. The inhibitory effect of penicillamine on C4A is 10-fold greater than that observed with C4B. At therapeutic doses of penicillamine (60 μM in plasma) 40% inhibition of C4A binding has been measured in vitro. Penicillamine contains both a sulphhydryl and an amino nucleophilic group but it has been demonstrated that it is through the sulphhydryl group in the drug that penicillamine becomes bound to the thiol-ester site in C4b. The covalent binding of penicillamine to C4 when C4 is activated is likely to occur through nucleophilic attack at the thiol-ester carbonyl group (Fig. 2, route E) rather than through formation of a disulphide bridge with the thiol-ester sulphhydryl group [41]. The concentration over which inhibition of C4A by penicillamine occurs falls within the therapeutic range and therefore it may be that inhibition of the C4A component contributes to the therapeutic effect of penicillamine in rheumatoid arthritis. In support of this suggestion, it has been shown that treatment of patients with penicillamine leads to a decrease in the deposition of C3b in rheumatic joints [56].

As with the toxic side-effects of the other drugs which have been described, penicillamine-induced immune-complex-mediated disorders affect a subpopulation of all patients taking the drug [57]. Patients who have a poor capacity to oxidize the sulphhydryl group of penicillamine are at increased risk of experiencing toxic side-effects, particularly side-effects involving problems of immune complex handling [54]. This is in accordance with the role suggested for inhibition of C4 in generation of toxicity proposed here in which the sulphhydryl form of the drug is required for inhibition of C4 in penicillamine toxicity [57]. A null allele for C4A is found in linkage disequilibrium with Dr3 but the importance of the C4 type in penicillamine-induced immune complex disorders remains to be assessed.

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