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The Role of Hypersensitivity and the Immune Response in Influencing Susceptibility to Metal Toxicity

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The immune status of the individual is an additional variable which has to be taken into account in any consideration of factors which influence the metabolism and toxicity of metals. The commonly occurring phenomena are described resulting from increased cellular reactivity to platinum, mercury, gold, nickel, chromium, and beryllium, and an attempt has been made to classify these into the four types of immune response. The clinical effects can be very varied, giving rise to conjunctivitis, rhinitis, asthma, urticaria, contact dermatitis, proteinuria, nephrotic syndrome or blood dyscrasia. Of these effects, cutaneous hypersensitivity is the most common, affecting both industrial and general population groups. Metal compounds used in therapeutics and metals used in prostheses have also been responsible for hypersensitive reactions.

In any consideration of factors influencing susceptibility to metal toxicity, the reactivity of the host has to be taken into account. Host reactivity influences both dose–effect and dose–response relationships. An effect may appear idiosyncratic, that is, untoward, or one not usually associated with the exposure. Alternatively, the usually associated effect may appear at a much lower dose level than that seen in the population, thus influencing the dose–response relationship. Such idiosyncratic phenomena may result from a variety of causes, such as for example, a variation in bioavailability which may be due to genetically determined enzyme deficiency. Idiosyncrasy may also result from allergy, a state of altered reactivity dependent on prior exposure. This term was originally used to denote both antibody-mediated immunity and also increased reactivity or hypersensitivity, and is now used mainly in the latter sense. Terminology remains confused, however, for hypersensitivity can be used, as here, to describe the increased reactivity resulting from the immune response or in a more general way to denote an untoward response from whatever cause. Substances capable of provoking an immune response are complex molecules. A simple molecule, such as a metal, requires complexing with protein, nucleic acid or polysaccharide in order to develop antigenic properties, i.e., to become capable of stimulating an immune response. This involves the formation of covalent bonds to form a hapten–carrier conjugate. Clinical observation has shown an individual predisposition to a hypersensitive reaction following exposure to a potential allergen. Animal experimental studies provide some evidence that the ability to become sensitized is genetically controlled, and inheritance as a single mendelian dominant has been demonstrated with mercury, beryllium, and chromium.

Of two inbred strains of guinea pig, 80% of one strain could be sensitized to mercuric chloride, while sensitization to potassium dichromate or beryllium fluoride did not occur. On the other hand, the second strain could not be sensitized to mercury, but over 70% could be sensitized to the other two metal salts (*1*). Thus the ability to become sensitized with these metals differs from strain to strain, and also within each strain.

Immune Responses

Immune responses which may be harmful to the tissues have been classified by Coombs and Gell (*2*) into four basic types. Essentially they can be considered as mediated either by the action of humoral

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antibodies or by specifically sensitized lymphocytes.

Type I: Anaphylactic or Immediate Hypersensitivity

The antibody, which is an IgE or reaginic immunoglobulin, reacts with the antigen on the surface of mast cells releasing vasoactive amines. Clinical reactions are varied, consisting of rhinorrhea, conjunctivitis, asthma, urticaria, or systemic anaphylaxis. Hypersensitivity can be transferred from a sensitive to a normal subject by means of serum, which contains the humoral antibody. The cutaneous, mucosal, and brochial reactions to platinum have been attributed to type I hypersensitivity although type III reactions may also be involved.

Type II: Cytotoxic Hypersensitivity

The humoral antibody, which is an IgG immunoglobulin, reacts with antigen or hapten bound to the cell surface and fixes complement to produce cell death. The thrombocytopenia produced by organic gold compounds is likely to be brought about in this way.

Type III: Immune Complex Hypersensitivity

The antibody combines with soluble antigen and the complex deposits in tissues, fixing complement and giving rise to a polymorphonuclear inflammatory response. The outcome is dependent on the relative proportions of antigen and antibody. With antibody excess, the complexes are rapidly precipitated, usually close to the site of origin of the antigen, to give rise to an Arthus reaction. With antigen excess, complexes are deposited in vessel walls by a filtering process. Granular or "lumpy" antigen–antibody complexes are deposited on the epithelial surface of the glomerular basement membrane in this way. This immune complex reaction is also responsible for the systemic reaction known as serum sickness.

Type IV: Cell-Mediated Hypersensitivity

The reaction, also known as delayed-type hypersensitivity, is mediated by thymus-dependent lymphocytes, taking 24–48 hr to develop in the sensitized individual compared with 15–30 min for anaphylactic and 4–8 hr for Arthus reactions. The type IV reaction consists histologically of a mononuclear response and is essentially similar to the Mantoux reaction. Delayed hypersensitivity can be transferred by the small number of specifically sensitized small lymphocytes present in a lymphocyte suspension. It is responsible for contact dermatitis following exposure to nickel and chromium. It has been suggested by Turk (*3*) that granuloma formation following exposure to zirconium and to beryllium may be a special type of cell mediated immune response.

Only certain metals have been shown to provoke an immune response, but the hypersensitive states to which they can give rise are important and in some cases very common. Mercury, gold, beryllium, platinum, nickel, and chromium have been chosen here to illustrate the variety of hypersensitive reactions which can be produced, but neither the metals nor the reactions described are exhaustive.

Platinum

Exposure to the complex salts of platinum such as ammonium tetrachloroplatinate or hexachloroplatinate give rise to an allergic reaction involving the skin, mucosae, or the respiratory tract in persons with no atopic tendency. The duration of exposure before sensitization occurs has been variable, ranging from a few months to many years, with a large proportion of exposed persons eventually developing symptoms.

Sensitized individuals present with conjunctivitis, rhinitis, asthma, urticaria, or contact dermatitis or with a combination of these. Anaphylactic reactions have also been reported following the administration of complex platinum salts as chemotherapeutic agents (*4*). Typical type I skin, nasal, and bronchial reactions have been elicited with platinum halide complexes, indicating the presence of mast cell sensitizing antibodies (*5*). The Prausnitz-Kustner reaction has also been demonstrated with passive transfer of sensitivity with the serum of a sensitized platinum worker (*6*).

Immediate, late and dual bronchial reactions were demonstrated by Pepys (*7*) in sensitized subjects exposed to dusts of complex salts of platinum mixed with lactose. The bronchial reactions, as measured by a fall in forced expiratory volume, could be inhibited by disodium cromoglycate. An Arthus type III reaction indicative of the additional presence of precipitating antibody has been demonstrated by Levene and Calnan (*8*). Levene (*9*) also postulated the formation of IgG antibody but was unable to demonstrate this, and further attempts to demonstrate the presence of IgE antibodies have not so far been successful (*10*). Cleare et al. (*10*) showed that the allergic response, as shown by skin tests with various platinum halide complexes, is

confined to a small group of charged compounds containing reactive ligand systems such as chlorine and to a lesser extent bromine, and is related both to their charge and their overall reactivity towards protein. Neutral complexes and those containing more strongly bound ligands were found to be inactive.

Mercury

Allergic reactions following exposure to inorganic or organic mercurials involve the kidney or the skin and are not uncommon.

Both proteinuria and the nephrotic syndrome have followed occupational and therapeutic exposure to mercury (*11*). Joselow and Goldwater (*12*) found an increased prevalence of proteinuria in mercury workers compared with a control group with a significant correlation between urinary mercury excretion and protein concentration. Kazantzis et al. (*11*) reported three cases of the nephrotic syndrome in a group of mercury workers, describing this as an idiosyncratic reaction, as the condition was not associated with other evidence of mercurialism or with urinary mercury concentration. Renal biopsy in two of their cases showed minimal lesions on light microscopy. Becker et al. (*13*), Cameron and Trounce (*14*), and Strunge (*15*) all found evidence of membranous glomerulonephritis on light microscopy. Using electron microscopy, Mandema et al. (*16*) demonstrated material laid down between epithelial cells and basement membrane proper, in one case without definite abnormality on light microscopy. Hilton, Jones, and Tighe (*17*) using electron microscopy also found widespread basement membrane thickening, and partial fusion of epithelial cell foot processes where light microscopy showed normal glomeruli.

Of 60 adult Africans with the nephrotic syndrome, 53% were using skin lightening creams containing mercury (*18*). In this series, exposure to mercury was associated with a minimal change glomerular lesion, present in half of the 34 patients subjected to renal biopsy, an unusually high proportion. However, 14 of these 17 patients gave a history of exposure to mercury. A few membranous and proliferative lesions were also seen in association with mercury exposure. In one case reported by Kibukamusoke, Davies, and Hutt (*19*) electron microscopy showed widespread foot process fusion with subepithelial and intramembranous deposits. Immunofluorescence showed finely granular IgG, IgM, and C3 complement deposits, indicative of a membranous glomerulonephritis with a likely immune complex pathogenesis. IgG and C3 complement deposits were reported by Lindqvist et al. (*20*) in eight cases where skin lightening cream had been used.

In the reported series of mercury-induced nephrotic syndrome, remissions occurred in a high proportion of the cases. However in two cases reported by Morel-Maroger and Verroust (*21*), clinical features persisted after cessation of exposure. The finding of electron dense deposits and of immunoglobulins in a few cases of mercurial induced nephrotic syndrome suggests that the lesions may be caused by the deposition of antigen-antibody complexes. More such studies need to be performed. The meeting on Maximum Allowable Concentrations of Mercury Compounds (*22*) referred to the nephrotic syndrome as occurring in rare cases, but the condition appears to be less uncommon than previously believed.

Contact dermatitis has been reported as occurring "sometimes" (*22*). However thiomersal and ammoniated mercury have been found to be common sensitizers in a survey performed on the Epidemiology of Contact Dermatitis (*23*), thiomersal being the third commonest sensitizer after nickel and chromium. Skin sensitization to mercury in amalgam dental fillings has been reported (*24*), and both aryl and alkyl mercurial seed dressings have been shown to be potent skin sensitizers. Cutaneous hypersensitivity to mercury as demonstrated by a standard patch test technique has been investigated in a large group of dental students (*25*). An increase in hypersensitivity was shown with length of exposure. Thus positive reactions were obtained in 2% of new entrants but in 18.8% of senior students, the increase through the years being statistically significant. No relationship was found between positive reactors and a history of previous skin allergy.

Gold

As with mercury, both proteinuria and the nephrotic syndrome have followed exposure to gold compounds, but in contrast, the reaction has only been seen following therapeutic administration of gold in organic form. While transient proteinuria is common, the nephrotic syndrome appears to be rare, only 18 cases having been reported up to 1970 (*26*). Inadequate documentation of earlier cases cast doubt on the causal role of gold given in rheumatoid arthritis, which itself may provoke the nephrotic syndrome in certain circumstances. However, recently carefully documented human cases and animal studies leave little doubt that gold can give rise to the nephrotic syndrome and that this is likely to be mediated through the immune response. In one series, proteinuria developed in five of 75 rheumatoid arthritics treated with gold and pro-

gressed to the nephrotic syndrome in two, although blood and urinary gold levels were similar to those found in 25 other patients without renal involvement (*27*). No difference was found in site or degree of gold deposition in tubular cells, interstitial tissue and glomerular tufts in cases with and without a nephrotic syndrome. Electron microscopy showed deposits on the epithelial aspect of the glomerular basement membrane as in membranous glomerulonephritis. Similar appearances have been noted by others (*28, 29*).

Renal biopsies from ten rheumatoid patients who developed proteinuria on gold therapy showed changes in seven of these characteristic of membranous glomerulonephritis (*30*). The ultrastructural features, together with the immunofluorescent appearances, indicated that complexes of immunoglobulin and of complement were involved. The appearances were identical with changes induced in rats following the injection of gold salts (*31*). In electron microscopic and immunfluorescent studies in two cases, one showed a typical membranous glomerulonephritis, while the other showed only minimal glomerular changes, but with substantial tubular degeneration. In the first case, electron microscopy revealed diffuse fusion of the foot processes of the glomerular visceral epithelial cells and subepithelial deposits. Immunfluoresence revealed deposits of IgG and IgM along the glomerular capillary walls. An immunological mechanism responsible for glomerular injury was postulated in the first, and a direct toxic effect in the second case (*32*). Further evidence in support of an immune complex disorder in gold nephropathy comes from serological tests on one patient (*33*). Tissue antibodies, circulating immune complexes and antimmunoglobulins were found which disappeared with the onset of the nephrotic syndrome, suggesting deposition in the glomeruli as immune complexes. Most reports agree that the renal lesion regresses once gold therapy is discontinued. Morel-Maroger and Verroust (*21*) reported regression in four of their five cases. No difference from so-called idiopathic membranous glomerulonephritis was demonstrable by histology or immunofluorescence. Gold is believed to give rise to nephropathy in one of two ways. It may damage the glomerular basement membrane releasing or altering material with the formation of antiglomerular basement membrane antibodies. Alternatively, gold may act as a hapten, combining with protein to form an antigen which then reacts with specific antibody immunoglobulins with subsequent deposition of these complexes on the basement membrane.

Gold also produces bone marrow depression with consequent abnormalities of the blood picture which have included thrombocytopenia, granulocytopenia and aplastic anemia. The absence of a correlation between the dose of gold and the degree of marrow depression suggests that a hypersensitivity reaction is involved. Evidence to this effect has been presented by Denman and Denman (*34*), who demonstrated lymphocyte transformation to blast cells following *in vitro* challenge with Myocrisin in six patients with bone marrow depression following gold therapy. Deren et al. (*35*) were unable to demonstrate an immunologic basis for gold induced thrombocytopenia with the lymphocyte transformation test or with a humoral antibody production test, although they, too, concluded that the disorder must be immunologically determined. In four of their six cases the thrombocytopenia was preceded by a skin rash, a sequence observed by others too.

Pulmonary fibrosis sometimes occurs as a complication of rheumatoid arthritis. However, more cases of pulmonary fibrosis have been reported among gold-treated rheumatoid patients than in those who have not had gold treatment. It has been suggested by Geddes and Brostoff (*36*) that in some cases this might be due to a hypersensitivity reaction to chrysotherapy. In the case reported by these authors, fibrosing alveolitis developed during a four month course of sodium aurothiomalate treatment, with remission and exacerbation of symptoms related to periods of gold therapy. Lymphocyte transformation was demonstrated, but there was no eosinophilia and serum IgE was normal. Winterbauer, Wilske, and Wheelis (*37*) described two cases of pulmonary fibrosis following gold therapy, both of which had eosinophilia, raised IgE levels and negative lymphocyte transformation.

Contact dermatitis may occur from metallic gold, but much more common is allergic dermatitis following organogold therapy. In one such series, the rash was preceded by eosinophilia and raised circulating IgE levels, which were also found in a proportion of treated patients without a skin reaction (*38*).

The evidence presented above shows that gold is likely to give rise, under appropriate circumstances, to types I, III, or IV hypersensitivity reactions.

Nickel

Nickel was found to be the most common skin sensitizer in patch testing studies performed on subjects suspected of having contact sensitivity (*23, 39*). Both occupational and general environmental exposure is common. There is at present no evidence that nickel hypersensitivity is responsible for damage to any tissue or organ other than the skin.

The type of immune reaction involved, as with most other forms of contact dermatitis, is type IV, T-cell-mediated or delayed hypersensitivity.

Nickel has been shown to act as a specific stimulant in patients showing nickel hypersensitivity when they are examined by the lymphocyte transformation test. Increased lymphocyte transformation occurs specifically in cells from nickel-sensitive subjects, but neither the sulfate nor the acetate was found to act in a nonspecific stimulating capacity (*40–42*). Forman and Alexander (*41*) also found that during the lymphocyte transformation process, lymphokines were liberated which temporarily inhibited the migration of macrophages. Leukocyte migration inhibition was demonstrated in both patch test positive and negative subjects with a history of nickel dermatitis (*43*). Significant inhibition was found only with nickel sulfate–albumin conjugate but not with the unconjugated form. Conflicting results have been obtained with the leukocyte migration inhibition test. Macleod, Hutchinson, and Raffle (*44*) found no statistical difference between the migration indices of nickel-sensitive and control subjects, contrasting with the positive results obtained in the same subjects with the lymphocyte transformation test. Jordan and Dvorak (*45*), using nickel sulfate without prior coupling to a carrier protein were able to distinguish subjects with nickel dermatitis from controls by inhibition of migration of polymorphonuclear leukocyte. They gave details on the technical requirements for the test. These authors refer to the complex nature of delayed hypersensitivity and point out that the various *in vitro* assays would only be expected to show a good correlation in an intact cell mediated immune system.

Hutchinson, Macleod, and Raffle (*46*) investigated the nature of the protein-hapten conjugate which could effect lymphocyte transformation. Radiochromatography identified binding to a number of amino acids, in particular to lysine, while autoradiography showed direct binding to the lymphocyte cell surface in both nickel sensitive and control subjects. They reasoned that the antigenic triggering of lymphocytes sensitized to nickel could result from (a) a conjugate formed with amino acids or serum proteins; (b) direct binding to lymphocytes, or (c) reaction with a serum component to produce an unknown factor which is the sensitizer. Katz et al. (*47*) estimated the nickel content of hair, but did not find this to be significantly different in nickel-sensitive subjects as compared to nonsensitive controls and concluded that hair could not be used as an indicator of sensitivity.

Chromium

Hypersensitivity to chromium as shown by the patch test with potassium dichromate was found to be second only to hypersensitivity to nickel as the most common form of skin sensitization in the studies quoted above, both occupational and general environmental exposures being responsible. It is likely to be the most frequently occurring occupational dermatosis. The evidence suggests that the hypersensitivity to chromium concerns chromium in its role as a hapten conjugated to protein rather than any specific chromium compound. The results of skin tests have shown that the hypersensitive state is dependent more on the solubility and penetration of the chromium compound than on its valency, threshold concentrations for hexavalent chromium being lower than for the trivalent form (*48*). Hexavalent chromium, while showing good skin penetration, is taken up by erythrocytes and has poor protein-binding capacity, but trivalent chromium binds strongly and in a stable form to plasma proteins. The hypothesis that hexavalent chromium is converted to the trivalent form by sulfur-containing amino acids in the skin, followed by conjugation with protein to produce the full antigen, is explored by Polak, Turk, and Frey (*49*). All chromium compounds tested give a positive response in the macrophage migration inhibition test. The skin reactive factor has been demonstrated following the incubation of sensitized guinea pig lymphocytes with chromium guinea pig serum conjugate, and it is this factor which is likely to be the mediator of the delayed type IV hypersensitivity reaction associated with exposure to chromium (*49*). Permanent desensitization in chromium-sensitized guinea pigs can be produced by intravenous injection of potassium dichromate followed by cutaneous application of a minimal amount. Furthermore, if the hapten is injected simultaneously or a short time before the sensitization procedure, permanent immunological tolerance can be induced. These phenomena can be explained by assuming that all specific antigen sensitive lymphocytes, both in the circulation and in the lymph nodes, have been inactivated or destroyed, tolerance therefore being acquired according to the clonal selection theory of Burnet (*50*). The desensitization process in guinea pigs may be accompanied by a "flare up" reaction consisting of an inflammatory response in sites where this had occurred previously. The experimental evidence suggests that this reaction is not dependent on cell mediated immunity but on other factors, possibly

related to the production of antichromium humoral antibodies, with similarities to the Arthus phenomenon. The theoretical implications of desensitization, tolerance, and the flare up reaction are discussed by Polak, Turk, and Frey (*49*).

An increased incidence of cutaneous hypersensitivity to chromium, and also to cobalt and to nickel, has been observed in patients with metallic orthopedic prostheses. Failure of the prosthesis due to loosening has been found to occur more frequently in such metal and particularly in chromium sensitive patients. It has been suggested that particles released from metal–metal prostheses are responsible for sensitization (*51, 52*).

Beryllium

Exposure can give rise to acute tracheobronchitis, pneumonitis, contact dermatitis, or to chronic beryllium disease. The latter is usually a multisystem disorder principally affecting the lungs. A dose–effect relationship between exposure and development of the chronic disease has not been demonstrated and the evidence suggests that this is an autoimmune disorder (*53*). Contact dermatitis following exposure to beryllium compounds is of the delayed form and likely to be due to T-cell-mediated hypersensitivity. Patients with chronic berylliosis have been shown to have a depressed sensitivity to tuberculin when compared with a matched control group (*54*). Hypergammaglobulinemia is frequently found (*55*) due principally to an increase in IgG levels. Such in increase has also been found in patients with acute berylliosis, beryllium dermatitis and in beryllium workers with no evidence of disease. Patients with beryllium dermatitis usually react positively to a patch test with a soluble salt, but may in addition develop a granuloma at the test site. Subcutaneous granuloma may also develop following patch testing in chronic beryllium disease (*56*).

The availability of the beryllium ion determines the intensity of skin hypersensitivity. Krivanek and Reeves (*57*) showed in guinea pigs that beryllium acts as a hapten, a beryllium albuminate preparation producing a stronger reaction than the beryllium ion, suggesting that beryllium guinea pig serum albuminate may resemble the true antigen. Passive transfer of hypersensitivity has been accomplished in guinea pigs with lymphocytes but not with serum (*58*). Both lymphocyte transformation and leukocyte migration inhibition have been demonstrated in beryllium sensitive subjects and in animal experiments (*59, 60*). However, the hypersensitive state may not correlate with the presence of berylliosis. Reeves (*53*) found in guinea pigs an apparent immunity to pulmonary berylliosis following previous sensitization with intradermal beryllium sulfate, and, furthermore, cutaneous hypersensitivity was suppressed following inhalation exposure. He postulated that the cellular response following cutaneous hypersensitivity may help to destroy an autoantigen formed in the lungs. The beryllium-induced macrophage migration inhibition test was found to be positive in two patients with chronic beryllium disease but not in others on steroid treatment. A proportion of beryllium workers also gave a positive response, while all controls, both normal and patients with sarcoidosis, were negative (*61*). The significance of sensitization in such workers, with regard to a disease which may first develop many years after exposure, is unknown.

Alekseeva, Vasil'eva, and Orlova (*62*), based on both clinical and rat experimental observations, postulated that the systemic lesions of chronic berylliosis with marked granuloma formation were conditioned by an autoimmune process probably connected with an endogenous factor. Antibodies were found to lung, liver, heart, DNA and RNA and these together with hypergammaglobulinemia and plasma cell proliferation in lymphoid and other organs were similar to the pattern seen in the collagenoses. Furthermore antithyroid antibodies were found in chronic berylliosis as in systemic lupus erythamatosus. Chronic berylliosis was seen as an immunologically determined disease of chemical etiology resulting from the abolition of natural tolerance to autologous proteins.

The noncaseating granuloma consisting of mononuclear cells together with occasional giant cells is seen in active chronic beryllium disease, zirconium granuloma, sarcoidosis and in the Kveim reaction. It is believed to be due to a special type of cell-mediated immune response (*3*).

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