Occupational asthma from nickel sensitivity: I Human serum albumin in the antigenic determinant

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ABSTRACT Occupational asthma from nickel sensitivity was confirmed in a male worker (SB) by allergy skin tests and inhalational challenge. In an ammonium sulphate coprecipitation test 63Ni was selectively precipitated from SB plasma indicating antibody with nickel related specificity. Preincubation of 63Ni with human serum albumin (HSA) increased the specificity of the coprecipitation test. Blocking experiments with nickel and copper(II) salts effectively inhibited the binding of 63Ni to antibody of SB plasma but did not affect control tests or that for antibodies to an unrelated antigen, ampicillin. Co2+ slightly inhibited the binding of 63Ni, while Zn2+ and Mn2+ failed to inhibit. This deactivation pattern corresponds to the known sequence of binding of these metals to the primary copper binding site of HSA. It is concluded that the antigenic determinant depends on the combination of Ni2+ with HSA at this specific copper/nickel plasma transport site.

Occupational asthma induced by low molecular weight substances is an increasing problem. The general features of the pathogenesis of asthma on an immunological basis due to IgE antibody are reasonably well understood.1 By contrast, asthma induced by reactive chemicals such as toluene diisocyanate (TDI) is poorly understood and most cases are unassociated with a detectable immune response.2 Most of these chemically reactive inducers of asthma are new to the human experience and, at least in part, account for the apparent increase in frequency of the disease. One possible explanation for the failure to account for the disease by the demonstration of antibodies is a use of inappropriate antigen preparations. McConnell et al were the first to document a case of asthma associated with the inhalation of nickel salts.3 Earlier, an incident of nickel related asthma due to exposure to nickel carbonyl had occurred in a patient with Löfller’s syndrome.4 More recently, two additional case studies have appeared,5 6 including our own.5 Generally, the patients exhibited dermatitis and a positive allergy skin test to nickel salts and responded to an inhalation challenge with nickel sulphate. McConnell et al concluded from skin and haemagglutination tests that circulating antibody to nickel was implicated.3 Our findings appear to confirm this conclusion, as the patient’s serum reacted in a Ni-HSA radioallergosorbant test for antibodies of the IgE class. In addition, in an ammonium sulphate coprecipitation (Farr)7 test with 63Ni, more label was precipitated from his plasma than control plasma specimens, further indicating the presence of antibody with Ni specificity.5 Coupling of Ni2+ with human serum albumin (HSA) appears to be required for antigenic activity. The correlative roles of Ni2+ and HSA in the antigenic determinant are explored in detail in this and a companion paper.8 Although occupational asthma due to antibodies with nickel related specificity is uncommon, the immunological principles evolved from our work should enhance our understanding of more common antibody dependent reactivity to metals, particularly to platinum.9 10

Binding of Ni2+ to HSA

Binding of Ni2+, including that in serum, to HSA, has been shown at physiological pH values.11 12 Autoradiography experiments in basic solution (pH 8·6) have shown that Ni2+ occupies the primary copper binding site of HSA.13 Judging from spectrophotometric data for the formation of the Ni-HSA complex8 14 and the magnitude (6 × 109 M−1, but see ref 14) of the corresponding association constant at pH 7·5, this mode of complexing is

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also important at pH 7.4.11, 15, 16

The primary copper binding site of HSA consists of a square-planar chelate ring formed by the N-terminus α-amino nitrogen, the first two peptide nitrogens, and the 3-nitrogen of the imidazole ring of residue 3.13, 15, 17 This site is common to several albumins including bovine, human, and rat.11, 18 Metal ion competition studies have shown that a threefold excess of Ni²⁺ suppressed the Cu²⁺ occupancy at this site to 5% (for comparison, the Cu²⁺ association constant is 9 × 10⁹M⁻¹, but see ref 14).13, 15, 19 By contrast, the same excess of Co²⁺ replaced only 50% of the Cu²⁺. Interestingly, Zn²⁺ did not compete with the Cu²⁺ and, like Mn²⁺, is suspected to have a different mode of attachment.15, 20–22 Because of these divergent affinities for HSA, metal ion competition studies were considered potentially applicable to the characterisation of the Ni²⁺/HSA/antibody interactions.

**Materials and methods**

**COLLECTION OF SAMPLES**

Serum and ACD plasma were obtained from a worker (SB) in whom occupational asthma from the inhalation of nickel sulphate had been proved by inhalation challenge.5 Control serum specimens were obtained from seven laboratory workers with no occupational exposure to Ni. Two ACD plasma specimens were obtained from the local Red Cross blood transfusion service; one was group A, Rh negative and the other was group B, Rh negative.

**EFFECT ON HSA OF THE ANTIBODY COPRECIPITATION TEST**

The ammonium sulphate ⁶³Ni/antibody coprecipitation (Farr) test included routine procedures.7, 23 The effect of preincubating the labelled Ni²⁺ with HSA was examined in the following manner. Twenty microlitres of HSA (1 mg/ml distilled water) or distilled water were incubated with 100 μl of ⁶³Ni, diluted 1/10 000 with borate-saline buffer (pH 8.3, 0.1 M H₂BO₃, 0.025 M Borax, 0.075 M NaCl) from prepared stock (⁶³NiCl₂, New England Nuclear, 7 × 10⁻⁴M Ni, 2mCi in 0.2 ml of 0.5 M HCl; and diluted to 5.0 ml with distilled water). The ⁶³Ni-HSA or control mixtures were incubated at room temperature for two hours and then 0.1 ml test serum was added. After overnight incubation at 4°C, 0.78 ml of the borate buffer and 1.0 ml of saturated ammonium sulphate were pipetted in. After centrifugation, the precipitate was washed with 50% (NH₄)₂SO₄, in borate-saline, and the radioactivity was then measured by liquid scintillation spectrometry after allowing the ⁶³Ni to stabilise in the counting cocktail overnight.23

**METAL ION COMPETITION STUDIES**

Blocking by metal ions of the ammonium sulphate ⁶³Ni/antibody coprecipitation test was studied using the following procedure. Twenty microlitres of the appropriate metal salt solution (10⁻⁶ to 10⁻²M in distilled water, or in the case of Cr³⁺ in 10⁻²M HNO₃) were incubated overnight at ambient temperature with 100 μl of plasma. Subsequently, 120 μl of a ⁶³Ni/HSA mixture (10 ml of ⁶³Ni diluted in borate-saline buffer at pH 8.3 incubated for two hours with 2 ml of 1 mg/ml HSA) was added. After overnight incubation at 4°C, the (NH₄)₂SO₄ coprecipitation test was carried out as before.

**Results**

Preincubation of ⁶³Ni with HSA before addition to SB serum, in the ammonium sulphate coprecipitation test, had little effect on the precipitation of ⁶³Ni (fig 1). This contrasts with the major reduction in the precipitation of ⁶³Ni from control sera.

Inhibition by nickel, copper, and cobalt salts of ⁶³Ni coprecipitation with SB plasma is illustrated in fig 2a. On a molar basis, NiSO₄ had the most potent blocking effect followed by CuSO₄ and then Co(NO₃)₂. In separate experiments NiCl₂ and Ni(NO₃)₂ were shown to be comparable with NiSO₄. From experiment to experiment, the absolute counts for SB and control samples varied slightly due to
Antibody with Ni\(^{2+}\) specificity reacts with Ni\(^{2+}/HSA\) complex

**Discussion**

EFFECT OF HSA ON THE ANTIBODY COPRECIPITATION TEST

The observed reduction (fig 1) in binding of labelled Ni to normal human sera as a consequence of prior addition of HSA denotes reduced availability of the \(^{63}\)Ni\(^{2+}\) for non-specific protein binding in the co-precipitation test. This decrease would also apply to SB serum. Thus the relatively small change in counts actually observed with SB serum is taken to indicate a concomitant equalising increase in specific antibody binding by this antibody containing serum. The quantity of HSA added to the labelled Ni before incubation with serum corresponded to 1/200 the quantity of HSA present in the test serum aliquot. Consequently, it may be concluded that the preformation of the \(^{63}\)Ni\(^{2+}/HSA\) complex, rather than simply an HSA concentration increase was responsible for the enhancement of interaction of \(^{63}\)Ni with antibody. Data on the pH dependence of the coprecipitation test for SB plasma reported in part II confirm this; they parallel that for the formation of the primary Ni\(^{2+}/HSA\) complex.\(^8\) On a more practical note, the use of HSA preincubation improved the specificity of the coprecipitation antibody test and was subsequently incorporated into the procedure.

METAL ION COMPETITION STUDIES

Excellent agreement was observed for SB plasma between the blocking effectiveness by metal ions and their known affinity for the primary copper binding site of HSA. There was blocking by Ni\(^{2+}\), Cu\(^{2+}\), and Co\(^{2+}\) but not by Zn\(^{2+}\), Mn\(^{2+}\), and Cr\(^{3+}\). Furthermore, there was no anion (Cl\(^{-}\), NO\(_3\)^{-}, SO\(_4\)^{2-}) dependence. Metal ion or anion inhibitory effects were not observed for control samples. Ni\(^{2+}\) and Cu\(^{2+}\) had no effect on labelled antigen binding in an ammonium sulphate coprecipitation test of serum with antiampicillin antibodies performed with labelled HSA-ampicillin reagent (see part II).\(^8\) These observations show that the antigenic determinant recognised by antibody in SB plasma required the occupation of the primary copper binding site of HSA by Ni\(^{2+}\). When Ni\(^{2+}\) was displaced but remained in the reaction mixture binding to antibody diminished.

**Ni/HSA complex in the antigenic determinant**

The patient's sensitivity therefore seems to depend on the interaction of Ni\(^{2+}\) with a native binding site on HSA and would appear to result from a unique host susceptibility. The molecular configuration of the Ni-HSA complex would presumably be "normal" but present in greater concentration in SB as a

settling of the protein precipitate in the liquid scintillation cocktail mixture. This process did not alter the shape of the inhibition or control curves.

The NiSO\(_4\), CuSO\(_4\), and Co(NO\(_3\))\(_2\) had no effect on the precipitation of \(^{63}\)Ni in tests with the two control plasma specimens. Moreover, these salts did not affect coprecipitation of an immunologically unrelated antigen with its corresponding antibodies. Thus, for the same concentration range, these salts produced no blocking of the antibody related coprecipitation of a labelled ampicillin-HSA reagent in the ammonium sulphate coprecipitation test.

Cr(NO\(_3\))\(_2\), ZnSO\(_4\), and MnSO\(_4\) had no effect on the precipitation of \(^{63}\)Ni from SB antibody containing plasma or from control plasma specimens (fig 2b).

![Graph](image-url)
result of the high occupational exposure to soluble nickel salts. Plasma Ni\textsuperscript{2+} concentrations are known to be enhanced in workers exposed to nickel in electroplating processes.\textsuperscript{24,25} The greater inhibitory effect of non-labelled Ni\textsuperscript{2+} compared with Cu\textsuperscript{2+} exhibited in fig 2a is surprising since the Cu\textsuperscript{2+}/HSA interaction is stronger by at least a factor of 15 (see association constants quoted above). This observation implies that the strength of the Ni\textsuperscript{2+}, HSA/antibody interaction depends on a special structural feature of the interaction of Ni\textsuperscript{2+} with HSA.

**IMPLICATIONS**

HSA was used as the binding protein in these experiments because we have knowledge of the specific binding site for Cu\textsuperscript{2+} and Ni\textsuperscript{2+}. In individuals reacting to other low molecular weight substances alternative host components might be more relevant, although in the case of the metal sensitiser platinum, the reactive RAST antigen was Pt reacted with HSA.\textsuperscript{19} Recently, a specificity of antibody for trimellitic anhydride (TMA)-modified secretory IgA has been postulated for individuals with TMA induced occupational asthma.\textsuperscript{26} Specificity for carrier HSA has also recently been reported for antibodies to TDI and TMA.\textsuperscript{27,28} This does not constitute interaction dependent on a normal physiological binding site on the protein comparable to the Cu\textsuperscript{2+} binding site on HSA. Nevertheless, TDI or TMA induced modification or denaturation could occur in vivo and represent the molecular basis for the stimulation of antibody and the resulting sensitisation. Thus alterations of host components may be on the basis of a normal binding site provided by the host or a denaturation produced by highly chemically reactive inhaled molecules such as TDI and TMA. Regardless, the recognition that the novel antigenic determinants yielded by these alterations can potentially be a basis of sensitisation may well usher in new approaches to the unravelling of the pathogenesis of reactions to environmental substances including those leading to occupational asthma. We are currently investigating the possibility that the requirement of the Ni/HSA complex for immunological reactivity could also apply to cellular immune responses operative in contact dermatitis and reactions to metal prostheses. The leukocyte migration test indicates that this may be so.\textsuperscript{28} Thus comparable mechanisms pertaining to antigenic specificity may well be involved in both humoral and cellular responses to nickel, and by analogy, presumably also to cobalt, platinum, and other metals. Clarification of these issues might be expected to improve the laboratory tests available to identify sensitised individuals.

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**References**

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