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 ^{63}Ni was selectively precipitated from SB plasma indicating antibody with nickel related specificity. Preincubation of ^{63}Ni 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 ^{63}Ni to antibody of SB plasma but did not affect control tests or that for antibodies to an unrelated antigen, ampicillin. Co^{2+} slightly inhibited the binding of ^{63}Ni , while Zn^{2+} and Mn^{2+} 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 Ni^{2+} 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 ^{63}Ni , more label was precipitated from his plasma than control plasma specimens, further indicating the presence of antibody with Ni specificity.⁵ Coupling of Ni^{2+} with human serum albumin (HSA) appears to be required for antigenic activity. The correlative roles of Ni^{2+} 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 Ni^{2+} to HSA

Binding of Ni^{2+} , 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 Ni^{2+} occupies the primary copper binding site of HSA.¹³ Judging from spectrophotometric data for the formation of the Ni-HSA complex^{8,14} and the magnitude ($6 \times 10^5 \text{M}^{-1}$, 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.^{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 Ni^{2+} suppressed the Cu^{2+} occupancy at this site to 5% (for comparison, the Cu^{2+} association constant is $9 \times 10^6 \text{M}^{-1}$, but see ref 14).^{13 15 19} By contrast, the same excess of Co^{2+} replaced only 50% of the Cu^{2+} . Interestingly, Zn^{2+} did not compete with the Cu^{2+} and, like Mn^{2+} , 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 Ni^{2+} /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 ^{63}Ni /antibody coprecipitation (Farr) test included routine procedures.^{7 23} The effect of preincubating the labelled Ni^{2+} 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 ^{63}Ni , diluted 1/10 000 with borate-saline buffer (pH 8.3, 0.10 M H_3BO_3 , 0.025 M Borax, 0.075 M NaCl) from prepared stock ($^{63}\text{NiCl}_2$, New England Nuclear, $7 \times 10^{-4} \text{M}$ Ni, 2mCi in 0.2 ml of 0.5 M HCl; and diluted to 5.0 ml with distilled water). The ^{63}Ni -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% $(\text{NH}_4)_2\text{SO}_4$ in borate-saline, and the radioactivity was then measured by liquid scintillation spectrometry after allowing the ^{63}Ni to stabilise in the counting cocktail overnight.²³

METAL ION COMPETITION STUDIES

Blocking by metal ions of the ammonium sulphate ^{63}Ni /antibody coprecipitation test was studied using the following procedure. Twenty microlitres of the appropriate metal salt solution (10^{-6} to 10^{-2}M in distilled water, or in the case of Cr^{3+} in 10^{-2}M HNO_3) were incubated overnight at ambient temperature with 100 μl of plasma. Subsequently, 120 μl of a ^{63}Ni /HSA mixture (10 ml of ^{63}Ni 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 $(\text{NH}_4)_2\text{SO}_4$ coprecipitation test was carried out as before.

Results

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

Inhibition by nickel, copper, and cobalt salts of ^{63}Ni coprecipitation with SB plasma is illustrated in fig 2a. On a molar basis, NiSO_4 had the most potent blocking effect followed by CuSO_4 and then $\text{Co}(\text{NO}_3)_2$. In separate experiments NiCl_2 and $\text{Ni}(\text{NO}_3)_2$ were shown to be comparable with NiSO_4 . From experiment to experiment, the absolute counts for SB and control samples varied slightly due to

Fig 1 Influence of HSA on coprecipitation of ^{63}Ni . Serum samples were incubated with preformed ^{63}Ni -HSA before ammonium sulphate coprecipitation test. Error bars shown correspond to relative standard error for two to six replicates calculated from variances of component terms.

Fig 2 Blocking of ammonium sulphate ^{63}Ni /antibody coprecipitation test by metal salts. Plasma samples were incubated with metal salt before addition of the ^{63}Ni -HSA complex. Coprecipitation test was carried out subsequently. (a) Inhibition by Co^{2+} , Cu^{2+} , and Ni^{2+} . As shown for NiSO_4 , Co^{2+} , and Cu^{2+} salts did not induce inhibition in control samples. (b) Lack of inhibition by Cr^{3+} , Zn^{2+} , and Mn^{2+} . As shown for MnSO_4 , Cr^{3+} and Zn^{2+} salts did not effect control plasma samples. Vertical bars denote standard errors corresponding to three replicate samples.

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 $\text{Co}(\text{NO}_3)_2$ had no effect on the coprecipitation 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.

$\text{Cr}(\text{NO}_3)_3$, 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).

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 $^{63}\text{Ni}^{2+}$ for non-specific protein binding in the coprecipitation 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 $^{63}\text{Ni}^{2+}$ /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.^{8,14} 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

result of the high occupational exposure to soluble nickel salts. Plasma Ni^{2+} concentrations are known to be enhanced in workers exposed to nickel in electroplating processes.^{24,25} The greater inhibitory effect of non-labelled Ni^{2+} compared with Cu^{2+} exhibited in fig 2a is surprising since the Cu^{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^{2+} , HSA/antibody interaction depends on a special structural feature of the interaction of Ni^{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^{2+} and Ni^{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.¹⁰ Recently, a specificity of antibody for trimellitic anhydride (TMA)-modified secretory IgA has been postulated for individuals with TMA induced occupational asthma.²⁶ Specificity for carrier HSA has also recently been reported for antibodies to TDI and TMA.^{26,27} This does not constitute interaction dependent on a normal physiological binding site on the protein comparable to the Cu^{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.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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