Human Antihapten Antibodies in Trimellitic Anhydride Inhalation Reactions

IMMUNOGLOBULIN CLASSES OF ANTI-TRIMELLITIC ANHYDRIDE ANTIBODIES AND HAPTEN INHIBITION STUDIES

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ABSTRACT Inhalational exposure to trimellitic anhydride (TMA) produces an immediate-type asthmatic or a late respiratory systemic syndrome in certain workers after a latent period of work exposure. TMA has been found to react with proteins to produce a hapten-protein complex (trimellitate [TM] protein) or become hydrolyzed in aqueous, alkaline solutions to produce a salt, NaTM. Using a solid-phase radioimmunoassay technique, antibodies of different Ig classes were detected against TM-protein conjugates. IgE antibody was detected in three of five workers with asthma. IgG and IgA antibodies were detected in most exposed workers but higher levels of antibody were found in symptomatic workers even after long periods without direct TMA exposure. IgM antibody activity against TM-human serum albumin (TM-HSA) was detected but did not differentiate symptomatic from asymptomatic workers. NaTM served as a hapten for study because it does not react with proteins to form a hapten-protein complex as TMA does. The NaTM only partially inhibited IgG antibody activity against TM-HSA and much smaller amounts of TM-HSA than of NaTM were required to neutralize IgG antibody. A similar result was found with TM-ovalbumin. The latter results suggest that some IgG antibody is directed against a TM-protein moiety, probably a TM-amino acid determinant. In contrast to IgG, marked inhibition by NaTM of IgA and IgM antibody against TM-HSA was found in the sera studied.

INTRODUCTION

The occurrence of a respiratory response to the inhalation of trimellitic anhydride (TMA)[1] during industrial exposure was recently described (1). The TMA was shown to be highly reactive with protein, rapidly forming a trimellitate (TM)-protein complex in vitro. Observation of the clinical characteristics of the workers with symptoms after TMA exposure showed that three types of respiratory responses occur. One type is consistent with immediate-type IgE-mediated asthma (TMA asthma), often with symptoms of rhinitis. The second type is a late onset respiratory systemic syndrome (LRSS) with cough, mucous production, occasional wheezing dyspnea and systemic symptoms of malaise including chills, myalgias, and arthralgias occurring 4–8 h after work exposure to TMA. A third response, occurring after high-dose exposure, appeared to be an irritant respiratory response with a cough as the predominant symptom. The TMA asthma requires a latent period of work exposure, is of immediate-type onset, and is associated with IgE antibodies demonstrated by skin tests and in vitro techniques. The LRSS requires a latent period of work exposure, there is no immediate-type skin reactivity, but IgG antibodies against TMA proteins have been demonstrated by in vitro tests. The irritant response requires no period of latent work exposure. Lymphocyte reactivity has been observed in all groups of workers (1). Individual workers varied in the severity of the clinical responses of the asthma and LRSS. Some workers could not continue to work in the TMA production unit, others could work there with the control of exposure to the TMA.

The TMA syndromes provide an opportunity for study of human antibodies against a hapten-protein complex where the protein in the clinical state is autologous protein. Systemic immunization of the workers is likely to occur through the inhalation of the TMA which com-

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[1]*Abbreviations used in this paper*: HSA, human serum albumin; LRSS, late respiratory systemic syndrome; NaTM, sodium trimellitate; OVA, ovalbumin; RIA, radioimmunoassay; TM, trimellitate; TMA, trimellitic anhydride.

bines with respiratory secretory proteins which are then absorbed producing a systemic immune response, a local immune response, or both.

The occurrence of TMA inhalation disease provides an opportunity for correlation of in vitro immunologic studies with clinical states. The current studies were done to determine whether we could detect antibodies of four Ig classes against the TM haptenic determinant using a solid-phase radioimmunoadsorbent technique which has been used successfully in analysis of antibody activities in sera of patients with hypersensitivity lung disease (2–4). Further, in these studies, some characteristics of hapten inhibition of IgG anti-TMA have been described.

METHODS

Sera. Serum samples were obtained from workers exposed to TMA who were interviewed by a nurse and physician (1). These workers were skin tested with TM-HSA and classified as having TMA asthma, LRSS, or both or as workers exposed to TMA without symptoms in relation to TMA exposure (except for an occasional irritant exposure episode). Sera were stored at −20°C until use. Control sera were from normal laboratory personnel with no TMA exposure. Sera used in these studies are from workers shown in Table I.

Antigens. TMA may be covalently linked to proteins to produce a complete antigen containing TM determinants. The conjugates made were TM-human serum albumin (HSA) and TM-ovalbumin (OVA) and they were prepared by the same procedure using the appropriate protein carrier. The protein at a concentration of 5 mg per ml in 9% sodium bicarbonate was chilled in an ice bath which was placed on a magnetic stirrer. Dry, finely powdered TMA, 5 mg for each milliliter of solution, was added gradually over approximately 10 min. Stirring was continued for another 50 min. The solution was then dialyzed first with 9% sodium bicarbonate and then several changes of distilled water until the dialysate had an OD at 240 nm of 0.05 or less.

The composition of the dialyzed conjugate was estimated from the concentration of the TM determinant and of the protein (1). Since sodium TM (NaTM) and TM-protein conjugates both have an absorption maximum near 240 nm, the OD of the conjugate at 240 nm was assumed to be proportional to its TM concentration after subtracting a correction for the absorption of the protein carrier at this wavelength. For HSA, the correction amounts to 1.7 times its OD at 280 nm, which is an absorption peak for most proteins. NaTM has a small absorbance at 280 nm (~16% of its absorbance at 240 nm) which can be corrected for by use of simultaneous equations (1) but its effect is negligible. The molar concentration of the TM determinant was then calculated from the corrected OD_{240} by dividing by the molar extinction coefficient for NaTM, 11,400. Since no precipitation occurred during conjugation, the concentration of the protein was presumed to be 5 mg per ml except for adjustment for the volume change in dialysis and was converted to molar concentration using the molecular weight of the appropriate carrier. The ratio of the molar concentrations of the TM and carrier then define the composition of the conjugate. This calculation assumes as a first approximation that the extinction coefficient of the hapten is unchanged by conjugation which could lead to an error in absolute magnitude but should allow detection of changes in ratio produced by altering the conditions of preparation. Several of these preparations were made before and during this investigation and they were all estimated to contain ~30 TM residues per HSA molecule.

NaTM was prepared by allowing TMA to dissolve in 9% sodium bicarbonate, pH 8.8, at room temperature. The pH of the solution was then adjusted as required. Alternatively,

TABLE I

Source of Serum Samples from Workers Exposed to TMA with Asthma, the LRSS, or Both or No Symptoms after Exposure to the TMA Unit

Worker	Clinical response* to TMA exposure	Direct work‡ exposure to TMA	Comment
1	Asthma	5 yr previously	Unable to work in TMA production§
2	Asthma	1 yr previously	Unable to work in TMA production
3	Asthma	1 yr previously	Serum IgA, 38 mg/dl; IgM, 36 mg/dl
4	Asthma and LRSS	Current	Able to work in TMA production with careful control of exposure‖
5	Asthma and LRSS	3 yr previously	Unable to work in TMA production
6	LRSS	5 yr previously	Serum IgA, 36 mg/dl
7	LRSS	Current	Absent serum IgA; IgM, 37 mg/dl
8	LRSS	Current	
9	LRSS	Current	Serum analysis done before clinical diagnosis
10	None	Current	Work exposure at least 2 yr to present
11	None	Current	Work exposure at least 2 yr to present
12	None	Current	Work exposure at least 2 yr to present
13	None	Current	Work exposure at least 2 yr to present
14	None	Current	Work exposure at least 2 yr to present

* Derived from reference 1.

‡ At time of serum sampling. Workers in plant continue to have low level TMA exposure although not directly in the TMA unit.

§ Applies to all symptomatic workers in plant but not in TMA production.

‖ Applies to all workers in TMA production who have symptoms to TMA exposure.

TMA may be hydrolyzed in water to form trimellitic acid but must be neutralized with sodium hydroxide since the free acid has very limited solubility.

Antibody analysis. The analysis of antibodies against TM protein was conducted by use of the polystyrene tube radioimmunoassay (RIA) technique. This solid-phase RIA technique, initially developed to measure small amounts of immunoglobulins by Salmon et al. (5), was extended to detect antibodies against a variety of antigens. These have included IgE, IgG, and IgM antibodies against trichinella antigen in trichinosis (6) and IgG, IgA, and IgM antibodies against pigeon serum in pigeon breeders' disease (3). The current RIA studies use the techniques applied in previous studies. Briefly, the following reagents are added in sequence to the polystyrene tubes with appropriate washes and incubation periods (7): TM protein to form the solid-phase immunoadsorbent; HSA to saturate any free polystyrene-adherent surface sites; dilutions of patient or control serum; heterologous anti-Ig specific for the heavy chain of the respective human Ig under study; and finally ^{125}I-labeled human Ig of the same class as that against which heterologous antiserum is directed.

In these studies, anti-IgE was produced in rabbits against the Fc fragment of myeloma$_{PS}$ in our laboratory. Rabbit antiserum specific for the heavy chain of IgG, IgA, and IgM was obtained from Behring Diagnostics, American Hoechst Corp., Sommerville, N. J. Specificity of these antisera was confirmed by immunoelectrophoresis showing reactivity against the respective Ig only. IgE was a Sephadex G-200, Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J., fraction of IgE myeloma serum$_{DB}$. IgG was purified human IgG from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. IgA from myeloma serum$_{SP}$ was purified by Sephadex G-200 and agar zone electrophoresis. IgM was a Sephadex G-200 peak I preparation of serum from a patient with a monoclonal IgM gammopathy. These preparations may contain trace amounts of other immunoglobulins, but the specificity of the polystyrene tube RIA test resides in the specificity of the antiserums against the heavy chains of these immunoglobulins. Immunoglobulins were trace-labeled with ^{125}I using chloramine-T (8). Appropriate dilutions of patient serum to be added in step 2 of the RIA procedure were determined in preliminary experiments and control sera were tested at the same concentrations.

The antibody activity is indicated by counts per minute of Ig bound. The counts per minute bound by the same serum in this technique may vary in actual numbers particularly with different isotope labels, and the technique is best used for each serum with positive and negative control sera run simultaneously.

Inhibition analysis. After determination of antibody activity against TM protein, a dilution of serum showing significant antibody activity against the TM protein was preincubated with varying concentrations of the inhibiting agent for 24 h at 4°C. The polystyrene tube analysis described above was repeated and the inhibition calculated by the formula: percent inhibition = (counts per minute before inhibition − counts per minute after inhibition)/(counts per minute before inhibition) × 100.

Serum Ig analysis. IgG, IgA, and IgM were quantitatively measured by use of radial immunodiffusion plates (Meloy Laboratories Inc., Springfield, Va.)

RESULTS

IgG antibody activity against TMA. The results of the RIA tests done to detect IgG antibody against TMA are shown in Fig. 1. All of the workers with TMA asthma,

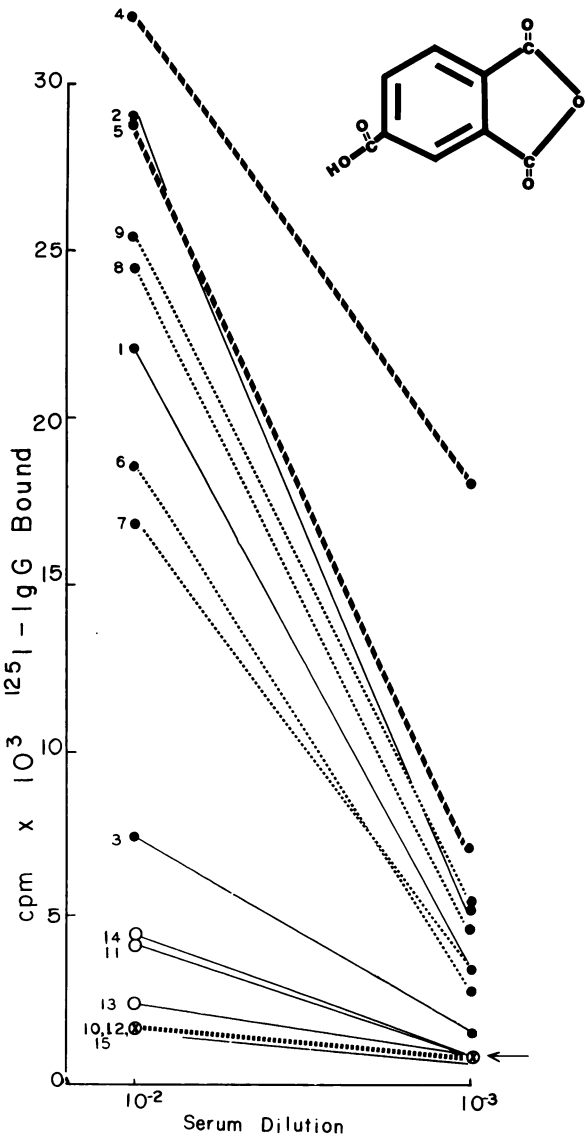

FIGURE 1 IgG antibody activity against TM-HSA detected by RIA of sera of workers exposed to TMA (Table I). TMA asthma (● —— ●), LRSS (● ---- ●), both responses (● - - - ●), asymptomatic workers (○ —— ○), nonexposed control (×---×). Control serum is also indicated by arrow. The structure of TMA is shown in the insert.

LRSS, or both had IgG antibody activity higher than that of workers currently exposed to TMA but with neither TMA asthma nor LRSS. One worker (no. 3 in Fig. 1) was not markedly different than asymptomatic workers. Symptomatic workers all demonstrated IgG antibody activity at a 1:100 and 1:1,000 dilution of serum. These antibody levels were significantly higher than those of the asymptomatic group at both 1:100 and 1:1,000 serum dilutions ($P < 0.0005$ and 0.01, respectively). Three asymptomatic workers demon-

strated antibody activity at a 10^{-2} dilution of serum. One worker (no. 9 in Fig. 1) had been initially reported as asymptomatic, was evaluated subsequent to these studies and found to have symptoms consistent with the LRSS.

IgA antibody activity against TM-HSA. 8 of 10 workers with asthma or LRSS, or both had IgA antibody activity above control levels or exposed asymptomatic workers (Fig. 2). The two workers who did not demonstrate IgA antibody activity against TM-HSA had either no demonstrable serum IgA or a low level of serum IgA (workers 3 and 7, respectively, Fig. 2 and Table I). Worker 6 also had a low serum level of IgA (Table I) and the lowest IgA antibody activity of all symptomatic workers demonstrating IgA antibody activity against TM-HSA (Fig. 2). One asymptomatic exposed worker (worker 10) demonstrated minimal IgA antibody activity against TM-HSA (Fig. 2). As a group, the symptomatic workers had significantly higher antibody activity than the asymptomatic workers at the 1:100 and 1:1,000 serum dilutions ($P < 0.01$ and 0.005, respectively).

IgM antibody activity against TM-HSA. Although four symptomatic workers with asthma, LRSS, or both had the highest IgM antibody activity against TMA (Fig. 3 and Table I), no other apparent correlation between symptomatic workers, asymptomatic workers, and current work exposure and IgM antibody activity is apparent (Fig. 4).

IgE antibody activity against TM-HSA. In three workers with asthma (workers 1, 3, and 5; Table I),

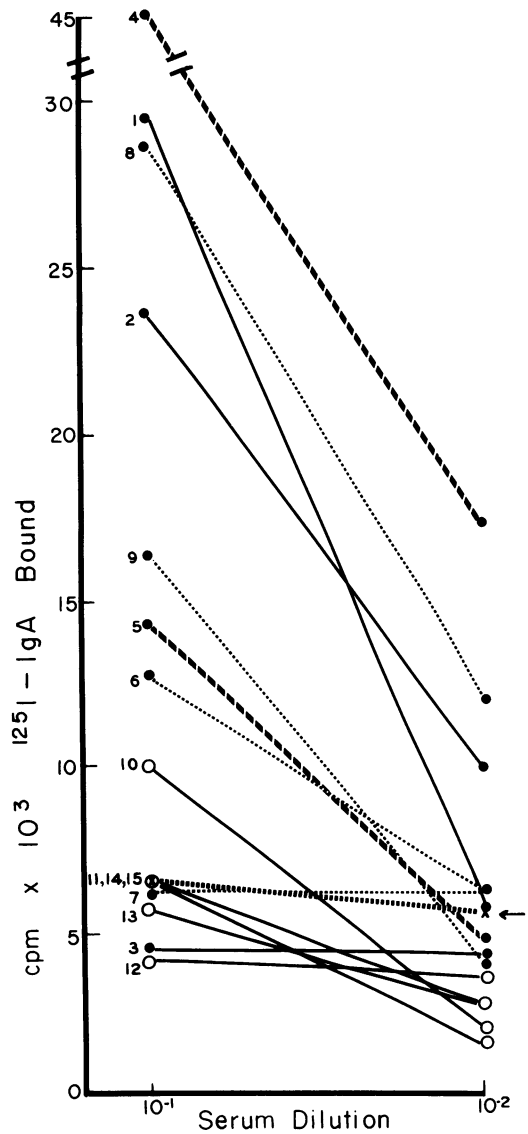

FIGURE 2 IgA antibody activity against TM-HSA detected by RIA of sera of workers exposed to TMA. TMA asthma (● —— ●), LRSS (●----●), both responses (● - - - ●), asymptomatic workers (○——○), nonexposed control (×----× and arrow).

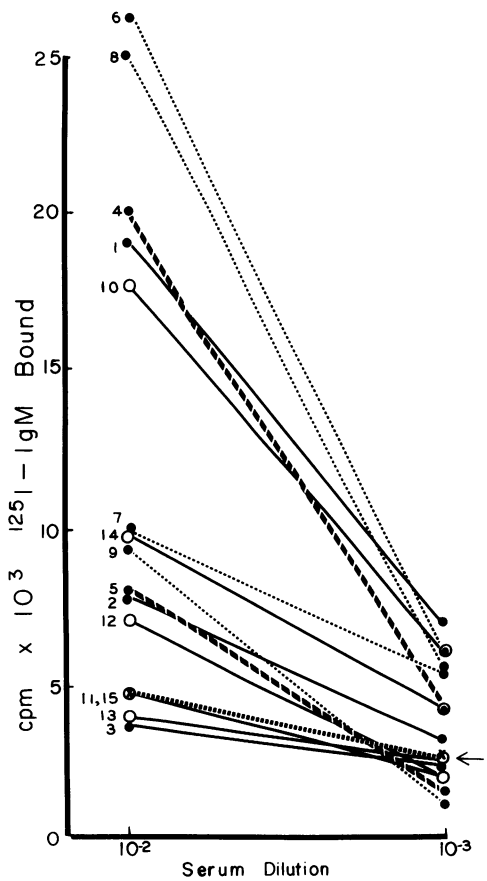

FIGURE 3 IgM antibody activity against TM-HSA detected by RIA using sera of workers exposed to TMA. TMA asthma (● —— ●), LRSS (●----●), both response (● - - - ●), asymptomatic workers (○ —— ○), nonexposed control (×----× and arrow).

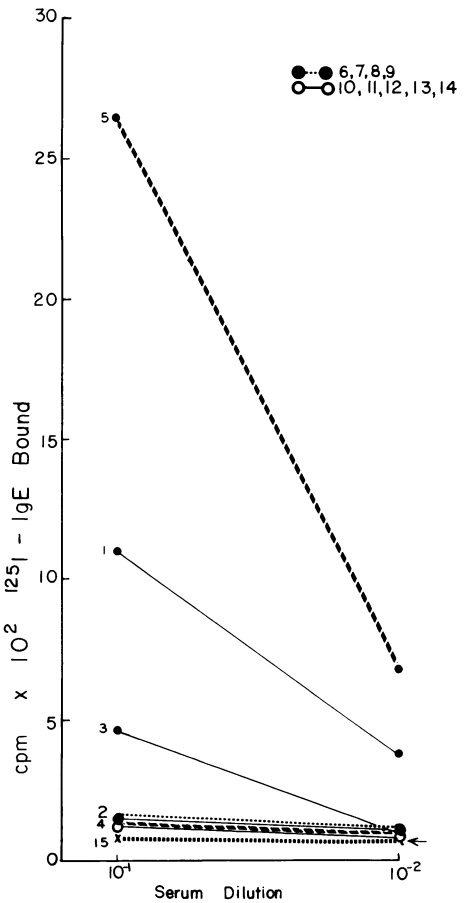

FIGURE 4 IgE antibody activity against TM-HSA detected by RIA using sera of workers exposed to TMA. TMA asthma (● —— ●), TMA asthma and LRSS (● - - - ●). All other workers are denoted by numbers.

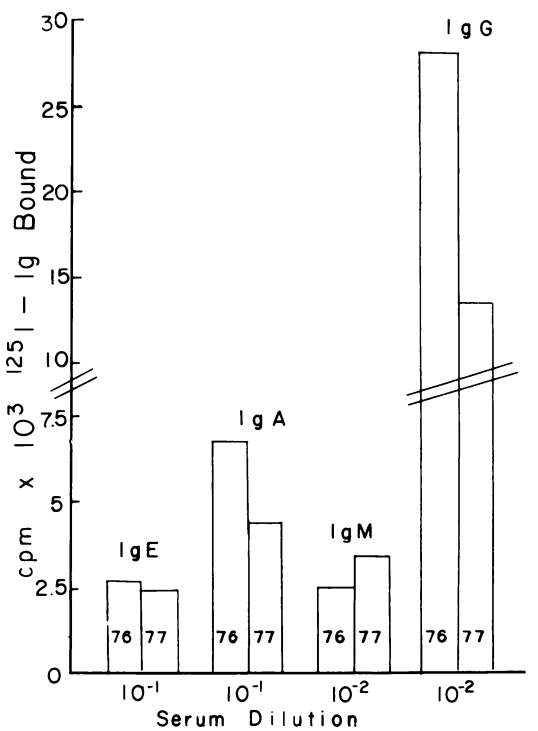

FIGURE 5 Anti-TMA-antibody activity in worker 5 (Table I). Serum samples were obtained in 1976 and 1977 at a 14-mo interval with no direct TMA exposure and antibody activity determined by the RIA at the same time. Antibody activity of the same Ig class but not different Ig classes may be compared in this graph. Control serum has been subtracted.

IgE antibody activity against TM-HSA was detected (Fig. 4). In workers 2 and 4 with asthma no IgE antibody was detected with the RIA. The latter two workers had high IgG antibody activity against TM-HSA and this IgG antibody may have inhibited the detection of IgE antibody against TMA. No other exposed workers demonstrated IgE antibody against TM protein whether they had LRSS or were asymptomatic.

Persistence of antibody activity against TM-HSA. Serum samples from worker 5 (Table I) were obtained in May 1976 and September 1977. IgG, IgA, IgM, and IgE antibody activity against TM-HSA was determined, running the paired samples simultaneously in the RIA. The results (Fig. 5) show a decline in IgG antibody activity but minimal or no differences in antibody activity of other Ig over the 14-mo period. This worker had not worked directly with TMA for 1½ yr before May 1976, but had remained in the plant where TMA is made.

Inhibition of IgG antibody against TM proteins by NaTM and TM proteins. IgG antibody activity against TM protein was used as a model system for studies of hapten inhibition. An appropriate serum dilution (determined from results of Fig. 1) demonstrating significant IgG antibody activity but not in great antibody excess was used. This dilution of serum was incubated with varying concentrations of either the hapten, NaTM, or the hapten-protein conjugate. After this preincubation, the standard technique was used to determine residual IgG antibody activity against TM-HSA. Preliminary experiments suggested that NaTM did not completely inhibit IgG antibody activity against TM-HSA and that considerably larger amounts of hapten as NaTM were required for equivalent neutralization as compared with hapten as TM-HSA. These results were confirmed in experiments of the type shown in Fig. 6A using serum of worker 4. As little as 0.04 mg TMA as TM-HSA completely inhibited binding whereas as much as 6–60 mg NaTM were only partially inhibitory (Fig. 6A). Using a different serum (worker 2) similar results were obtained and TM-OVA was as effective as TM-HSA in inhibition (Fig. 6B). Results of hapten or hapten-protein inhibition studies with several sera are listed in Table II. These results confirm that

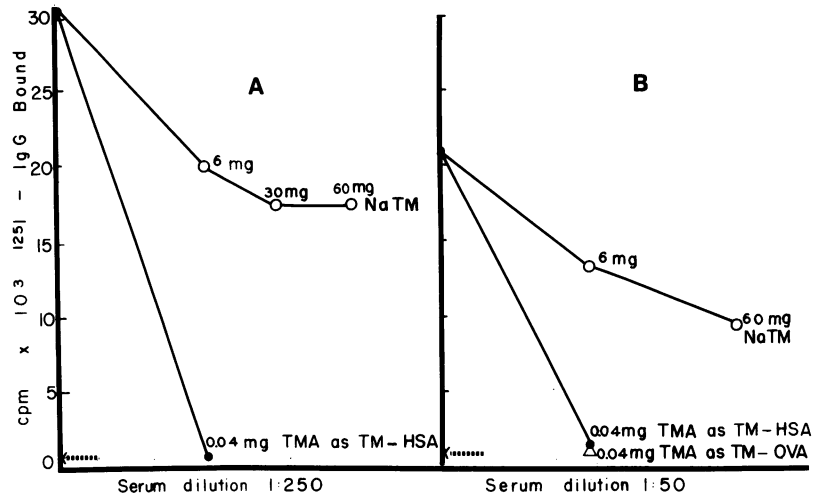

FIGURE 6 Hapten or hapten-protein inhibition studies using IgG antibody against TM-HSA as a model system. (A) a 1:250 dilution of serum of worker 4 (Table II and Fig. 1) was preincubated with TM-HSA or varying concentrations of NaTM before determining IgG antibody activity against TM-HSA. Complete neutralization of IgG antibody activity with TM-HSA but only partial inhibition with great excess of NaTM. (B) A similar experiment using a 1:50 dilution of serum of worker 2 (Table I and Fig. 1). Similar results with TM-OVA and TM-HSA are observed.

preincubation of serum samples with NaTM did not completely inhibit antibody activity against TM-HSA when compared with inhibition by TMA as TM protein even when NaTM was preincubated in great excess. The percent neutralization by NaTM varied with serum samples from different workers. Finally, TM-OVA was equivalent to TM-HSA in ability to inhibit IgG antibody activity under the conditions of these experiments.

Failure of IgG anti-TM–NaTM complexes to dissociate and react preferentially with TM-HSA. Serum of worker 4 (Table I, Fig. 1), diluted 1:250, was incubated in polystyrene tubes coated with TM-HSA for periods of 1, 24, 48, 72, and 96 h. Duplicate samples were incubated under identical conditions but in the presence of 60 mg/ml NaTM. At the end of the varying incubation periods the tubes were washed and anti-IgG and ^{125}I-IgG were added in the standard manner. This experiment was done to determine if anti-TM might dissociate from NaTM and preferentially react with TM-HSA. The results (Fig. 7) show that the IgG

TABLE II
Inhibition of IgG Antibody Against TM-HSA by Hapten or Hapten-Protein Complexes

Source of serum (worker)*	Percent inhibition of IgG antibody against TM-HSA by preincubation with hapten or hapten protein		
	TMA as TM-HSA (0.4 mg)	TMA as TM-OVA (0.4 mg)	TMA as NaTM (60 mg)
2	100	100	54
4	100	100	42
5	100	100	62
8	100	100	75

* See Table I and Fig. 1.

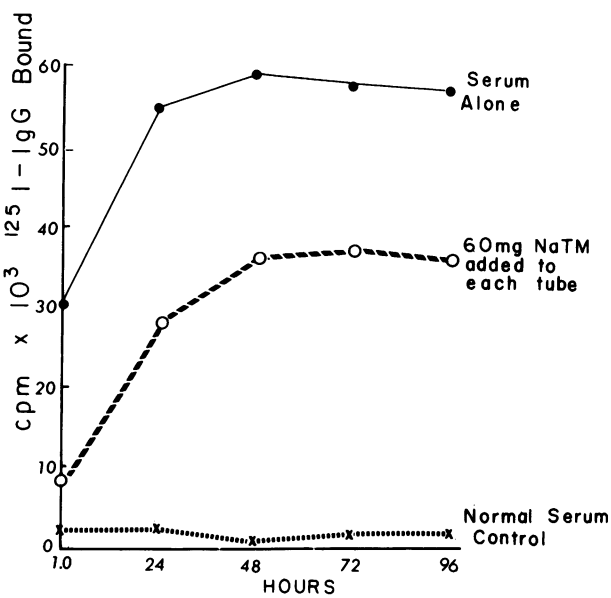

FIGURE 7 The effect of incubating IgG anti-TMA for various periods of time in TM-HSA-coated tubes in the presence of NaTM. Serum (1:250) from worker 4 (Table I incubated in TM-HSA-coated tubes for varying periods of time (●). The same serum incubated in TM-HSA-coated tubes in the presence of 60 mg NaTM (○). No discussion and preferential binding of anti-NaTM to TM-HSA is evident.

anti-TM remained neutralized by the NaTM. The 96-h incubation period showed a 37% inhibition which approximated the 42% inhibition of Fig. 6A. The experiment was repeated with the serum of worker 2 and the same type of inhibition pattern occurred. Thus, that portion of the anti-TM neutralized by NaTM did not dissociate from NaTM to react preferentially with TM-HSA.

Inhibition of IgA and IgM antibody against TM-HSA by NaTM and TM-HSA. The model system used to evaluate inhibition of IgG antibody against TM-HSA by NaTM or TM-HSA was applied to inhibition of IgA and IgM antibody against TM-HSA. Selected sera with high levels of IgA or IgM antibody activity against TM-HSA were studied. The results of these studies are summarized in Table III. Two of three sera with IgA antibody activity against TM-HSA were markedly inhibited by NaTM. Both sera with IgM antibody activity against TM-HSA were inhibited by NaTM. This is in contrast to the studies of IgG antibody against TM-HSA in which none of the sera could be completely inhibited by NaTM.

DISCUSSION

TMA reacts with various proteins to produce a TM-protein complex. This is demonstrated by the TM-HSA and TM-OVA prepared in these experiments. In production of TMA-immune responses, we suggest that inhaled TMA reacts with proteins of the respiratory secretions to produce TM-protein conjugates and a sufficient amount of these TM proteins are absorbed from the respiratory tract to result in a systemic antibody response against TMA and the TM-protein complex. The antibody response has been shown in the IgG, IgA, IgM, and IgE classes. The IgE antibody appears correlated with the rhinitis-asthma syndrome in three of five subjects. The other two patients had high levels of IgG antibody to TMA which is capable of interfering with the detection or measurement of IgE antibody of this specificity. Serum IgG antibody activity was elevated in workers with asthma, LRSS, or both as compared with asymptomatic workers. IgA antibody activity was elevated in workers with respiratory syndromes except in two workers, one with absent serum IgA and one with a depressed serum IgA level (Fig. 2). IgM antibody activity was highest in four symptomatic workers but otherwise did not clearly separate symptomatic from exposed asymptomatic workers.

TABLE III
Inhibition of IgA and IgM Antibody Against TM-HSA by Hapten or Hapten-Protein Complexes

Source of serum (worker)*	Percent inhibition of IgA antibody against TM-HSA by preincubation with hapten or hapten protein	
	TMA as TM-HSA (0.4 mg)	TMA as NaTM (60 mg)
1	100	95
2	100	100
8	100	72
	Percent inhibition of IgM antibody against TM-HSA with hapten or hapten protein	
	TMA as TM-HSA (0.4 mg)	TMA as NaTM (60 mg)
1	100	100
8	100	100

* See Table I and Figs. 2 and 3.

Although the TMA reacts rapidly with proteins to form a hapten-protein complex, the sodium salt of TMA, NaTM, does not react with proteins. The NaTM was used as the hapten for studies of hapten inhibition of antibody against the TM-HSA. Unexpectedly, it was found that the NaTM did not completely inhibit IgG antibody activity against the TM-HSA complex, and that significantly less TMA as TM-HSA resulted in complete inhibition of antibody. Further, TM-OVA was as effective in neutralization of IgG antibody as TM-HSA. These results suggest that all IgG antibody is not directed against the hapten but a portion of the IgG antibody is directed against the combined determinant formed by the TMA and the protein. Because complete neutralization occurred with TM-OVA this suggests that the antibody directed against the determinant formed by the TM protein is against the amino acid with which TMA reacts. Although TM-HSA could be the actual immunizing hapten protein because HSA is a normal component of respiratory secretions capable of combining with TMA, OVA is not a respiratory protein. Thus, antibody directed against TM-HSA and TM-OVA must be against a restricted portion of the protein molecule such as a TMA-amino acid common to both TM-HSA and TM-OVA. A variety of studies of anti-hapten antibodies in animals are likely to be relevant to those anti-TMA antibodies in man which are not completely inhibited by NaTM alone. For example, it has been shown that antibodies against 2,4-dinitrophenyl as a haptenic determinant have specificities against the 2,4-dinitrophenyl amino acid residue to which it is attached, primarily lysine (9, 10). Those anti-TMA antibodies not neutralized by NaTM may be directed against TMA lysine and this possibility will be investigated in future studies.

The degree of inhibition of IgG antibody against TM protein by the hapten, NaTM, varies with sera from different workers (Table II). Further, the degree of inhibition of antibody against TM protein by NaTM may vary with the Ig class of antibody, as shown by the fewer number of sera in which IgG antibody activity was inhibited by NaTM (Table II) as compared

with inhibition of IgA and IgM antibody activity (Table III). Antibodies directed against the hapten protein may result in different biologic reactions than antibodies directed against the hapten alone. Inhaled TMA may react in airway secretions to produce TM protein or be neutralized to produce NaTM. Antibody neutralized by NaTM forming an antibody-TM complex might have different biologic reactivity than an antibody–TM-protein complex, with the latter being more likely to form antibody–hapten-protein aggregates.

The antibody activity against TM proteins is present for long periods of time after discontinuation of direct exposure in the TMA production unit as evidenced by antibody activity in workers who had stopped working with TMA for one or more years (Table I). Antibody levels changed minimally in some Ig classes in one worker studied at an interval of 14 mo with no direct TMA work exposure. Although some workers were not working directly with TMA, they remained in the plant where TMA was produced and may have received enough TMA exposure to maintain an immune response but insufficient to produce symptoms.

We suggest that the mechanisms by which TMA produces asthma and the LRSS are immunologic in origin. The latent period of exposure before occurrence of symptoms, the limited population involved and the antibody activity against TMA are all consistent with this. The IgE antibody is likely to be a major factor in the rhinitis-asthma syndrome. The LRSS is more difficult to define, and a number of possibilities exist. TM-protein–antibody complexes may be significant. Antibodies of several classes could combine with TMA which has reacted chemically with surface proteins of a variety of cells. Such cells could agglutinate or be lysed if complement were activated. Lymphocyte reactivity stimulated by TM protein or by antibody dependent cellular cytotoxicity (where the TMA has fixed to cell surfaces and antibody has reacted with the TM protein) are other mechanisms to be investigated.

The TMA respiratory syndromes and the antibody responses are of significance in the population of workers involved. Of further interest is that the analysis of immune mechanisms involved and methodology used to characterize the antibody response may be of potential use in study of other diseases where inhalants or ingestants combine with autologous proteins.

Further studies to define which TMA-amino acid conjugates are capable of inhibiting the antibody binding of TMA protein are planned.

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