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PRODUCTION AND CHARACTERIZATION OF HUMORAL IMMUNE RESPONSE AGAINST MUSTARD GAS

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ABSTRACT

One of the promising aspects of the immunological research on chemical war gas is to investigate the immunogenicity of some hazardous compounds such as mustard gas.

Mustard gas is categorized as a "hapten" based on its physical and chemical properties. Haptenic chemicals which do not possess immunogenicity could be immunogenic experimentally when conjugated with a suitable protein carrier. To do so, mustard gas was coupled to a protein carrier and injected to an animal model (rabbit). After hyperimmunization, specific antibodies were obtained through special purification procedures and used in different immunological tests. It was observed that there are two different groups of antibody populations, one against the haptenic group and the other towards the protein carrier.

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INTRODUCTION

Following the widespread use of chemical weapons by the Iraqi regime against Iranian combatants and innocent civilians of our country, it was everyone's duty to try to somehow minimize the harmful effects of chemical war gases. In this regard, the immunogenicity of chemical war gas such as mustard gas was investigated. An effective way to confront chemical war gases in order to reduce their late harmful effect is to amplify the body's defense mechanisms. Based on physical and chemical properties mustard gas by itself is not an immunogen, rather it is immunosuppressive when entering the animal or human body without any modification. In order to induce immune responses against mustard gas, which is considered as a hapten, it was necessary to modify the hapten into an immunogen by covalently conjugating the small molecule to a large and foreign protein carrier. In this study specific immune responses against mustard gas were induced and characterized using different immunological approaches and the results of some of these investigations are presented in this article.

MATERIALS & METHODS

A. Immunogen synthesis (mustard gas-protein conjugation):

Mustard gas was covalently bound to different protein carriers according to a modified procedure reported by Fleming[3] after some modification (under publication). The protein carriers used were: bovine serum albumin, BSA (Ortho or Sigma), keyhole limpet hemocyanin, KLH (Calbiochem) egg albumin, eggal (Sigma).

Conjugation procedures were proved to be successful using different experimental approaches (under publication).

B. Immunization and Bleeding:

After conjugation and characterization, the conjugates were injected to rabbits intrascapularly using complete Freund's adjuvant (Sigma). Hyperimmunization was acheived by consequent injections, done four times yearly. Bleedings were carried out two weeks before and after immunization and repeated in two week intervals before the next injection.

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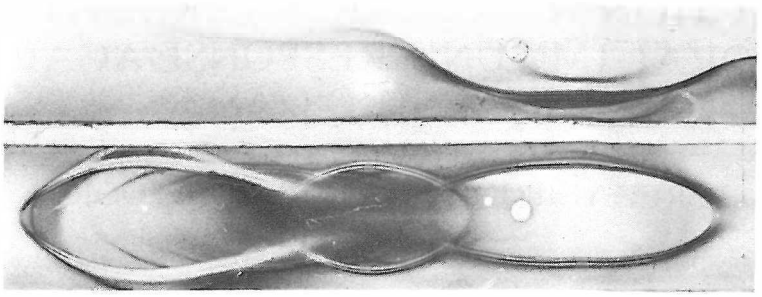


A. Purified Ig from DEAE sephadex
B. Normal rabbit serum
After electrophoresis, anti-rabbit whole serum antibody was added to the trough.

Figure 1. Immunoelectrophoresis.

C. Purification of serum immunoglobulins (Igs):

After removal of serum lipoproteins,[5] total Igs were also precipitated by $(NH_4)_2 SO_4$ (50%).[5] Anti-protein carrier antibodies were eliminated by incubation of total Ig preparations with the specific protein carrier. The resultant immune complex precipitates were removed by centrifugation (30 min. × 12000g). The IgG fraction was purified by anion exchange chromatography using DEAE-Sephadex A-25 (Sigma).[5]

D. Double diffusion test (D.D.T, Ouchterlony test):

To study the production and specifications of induced antimustard gas-protein carrier, the classic double diffusion test was performed. To do so, different conjugates of mustard gas (H) with different protein carrier were synthesized and used as test antigens. The conjugates used in the D.D.T. were BSA-H, KLH-H and eggal-H. The antibody preparations used in D.D.T. were commerical anti-BSA antibody, experimentally induced anti-BSA-H, anti-KLH-H antibodies.

RESULTS

A. Purification of Ig:

After removal of lipoproteins and albumin, the IgG fraction was purified. To prove the purity of the fraction immunoelectrophoresis was performed (Fig.1). In order to eliminate anti-protein carrier antibodies, the absorption test was used and the success of this elimination was proved by D.D.T. (Fig.2).

B. Double diffusion test (D.D.T):

To study the production and specifications of induced antimustard gas-protein carrier conjugates, the classic D.D.T. was performed using different test antigents (BSA; KLH; eggal; BSA-H; and eggal-H) (Tables I, II,III).

Table I. Double diffusion (Ouchterlony) test.

Test Antigens	Antibodies	
	Anti-BSA[a]	Anti-BSA-H[b]
BSA	+	+
BSA-H	+	+

a = Commercial antibody
b = H-Mustard gas
+ = Formation of precipitation line

Table II. Double diffusion (Ouchterlony) test.

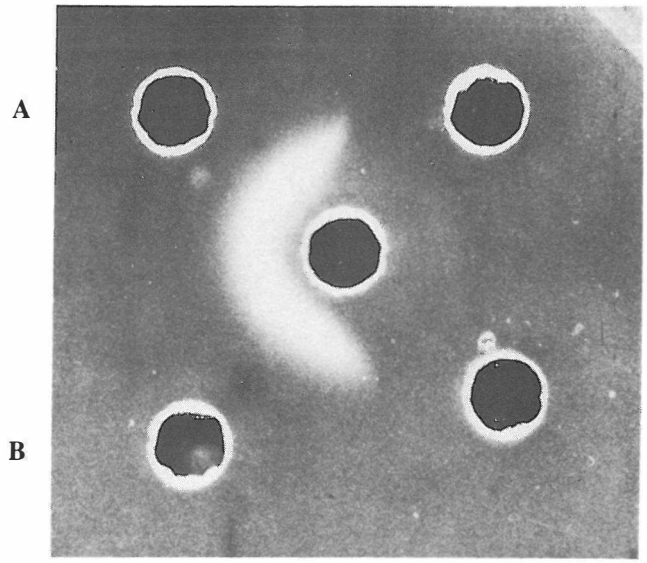
Test Antigens	An ibodies	
	Anti-BSA[a]	Anti-BSA-H[b]
Eggal.	–	–
Eggal.+H	–	+

a = Commerical antibody
b = H-Mustard gas
+ = formation of precipitation line
– = No precipitation line

Table III. Double diffusion (Ouchterlony) test.

Test Antigens	Antibodies	
	Anti-BSA[a]	Anti-BSA[b]
KLH	–	–
KLH-H	–	–*

a = Commerical antibody
b = H-Mustard gas
* = The absence of precipitation line is probably due to aggregation and therefore, perturbation of diffusion pattern in agar gel.
– = No precipitation line



Center well contained BSA,
A: Rabbit anti-BSA-H antibody,
B: Rabbit anti-BSA-H antibody,
C: Rabbit anti-BSA-H antibody,
after absorption with carrier,
D: Rabbit anti-BSA-H antibody after absorption with carrier.

Figure 2: Double diffusion test

DISCUSSION

The vesicant, mustard gas, has immediate harmful effects on different organs such as the eyes, skin, gut, etc. It may also have some late harmful effects on different internal organs. It has also been shown that this alkylating agent has some carcinogenic and mutagenic effects.[1,2,4]

One way to confront this chemical agent in order to reduce its late harmful effects is to amplify body defense mechanisms. To do so, it was necessary to induce specific immune responses against this toxin.

Based on physical and chemical properties of mustard gas, this chemical agent is not an immunogen by itself and is considered as a "hapten". To convert a hapten to an immunogen, it is necessary to covalently conjugate the small molecule to a large protein carrier.

In this study, mustard gas, as a hapten, was covalently bound to different protein carriers such as BSA, KLH, and eggal., and injected intrascapularly to rabbits. After hyperimmunization and bleeding, total Ig or IgG fraction were purified from prepared sera. The purity of IgG fraction after anion exchange chromatography was confirmed by immunoelectrophoresis (Fig.1).

To eliminate anti-protein carrier antibodies, the absorption method was applied. In Fig. 2, double diffusion test was carried out in order to show the removal of anti-carrier antibodies. As it is shown in parts C and D of this figure, there is no precipitation line between absorbed serum and protein carrier wells. To show the induction and reactivity of antibodies against haptenic and carrier portions of an immunogen, double diffusion (Ouchterlony) test was performed (Tables I, II, III). As it is shown in these tables, the induced antibodies are directed specifically towards both hapten and carrier. To prove that after conjugation, there was not any antigenic change in the protein carrier, the induced sera and commercial anti-BSA antibody were tested with BSA (intact) and BSA-H (conjugate). As it was shown in Table I, induced anti-BSA-H antibodies and commercial anti-BSA antibody both reacted with BSA alone and BSA-H conjugate. This finding indicates that there was no significant change in the protein carrier after conjugation procedures.

To show the production and specificity of anti-haptenic antibodies induced by BSA-H injections, a protein carrier conjugate which did not have cross reativity with BSA should be used as a test antigen in the double diffusion test. To accomplish this, eggal-H and KLH-H conjugates were used to detect anti-mustard gas (H) antibodies induced by BSA-H injection (Table II, III). As was shown in Tables II and III

there was no cross reaction between BSA and eggal. or KLH, whereas the presence and the specificity of anti-H antibodies were proved by these measures.

Finally this study clearly shows that we can induce specific immune responses against chemical was gases (i.e.mustard gas). Based on this finding, it is concievable to manufacture new vaccines against chemical war gases or to produce therapeutic sera, especially via monoclonal hybridoma technology.

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