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Original Articles

EVALUATION OF SERUM IMMUNOGLOBULINS AND THEIR RELEVANCE TO CHRONIC LUNG DISEASE IN MUSTARD GAS VICTIMS

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ABSTRACT

Despite the fact that 8 years has elapsed since the end of the imposed war, chemically injured victims are still suffering from various long-term complications, most of them respiratory in nature. The progressive nature of respiratory problems in these patients suggests that the immune system must be involved to initiate a cascade which ends up causing lung injuries.

To evaluate this, humoral immunity was assessed in 179 mustard gas victims in 1992. The patients were followed up for the next 4 years. These included 172 male and 7 female patients with a mean age of 33 years and an average of 6.1 years post-exposure to mustard gas. After physical examination and spirometric evaluation, these patients were divided into 3 groups based on the severity of their respiratory problems, i.e."severe" (group 1), "moderate" (group 2) and "mild" (group 3). IgG, IgA, C3 and C4 were measured using SRID technique and IgE by ELISA. The results in each group were compared with the control group consisting of 49 healthy, randomly selected volunteers. Mean age was 32 years in this group.

The results indicate that there is a significant fall in IgG, IgA, C3 and C4 levels in group 3 (p=0.009, 0.01, 0.004, and 0.002, respectively) as compared to the control group. IgG had also dropped significantly in groups 2 and 1. On the other hand in group 3, 19.6% and 15.2% of patients had lower than normal IgG and IgA levels, respectively. In group 2, 7.7% of patients had low IgG and 5.8% had low IgA levels. These figures were 10% and 3.8% for IgG and IgA in group 1. Of a total of 7 patients in group 3 with low IgA levels, four patients also had low IgG levels.

Two of these patients gradually developed more serious rspiratory problems and were classified as group 1 after two years.

We concluded that: 1) mildly injured patients (group 3) have lower IgG and IgA as well as C3 and C4 levels, and 2)among these patients those who demonstrate low IgG levels are more prone to develop progressive respiratory problems in the future.

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Serum Immunoglobulins in Mustard Gas Victims

INTRODUCTION

Mustard gas was first synthesized in Germany in 1854 by Victor Meyer. It's blister forming properties were discovered in 1887, and it was used as a weapon during World War I by German forces against French and Canadian forces near Ypress, Belgium in 1917.[1] Vesicants are used as a weapon in mainly 2 forms: "yprite" and "lewisite". These are alkylating agents capable of reacting with target molecules by strong electropilic reactions through formation of sulfonium ion or transitional complexes. Thus they can react with phosphates, amines, sulphydrils, hydroxyls and imidazole groups. They can also react with DNA and thus interfere with vital cellular activites and bring about cell death.[2] Nitrogen mustard (e.g. cyclophosphamide) is used as an antitumor agent.

During the imposed war, Iraq massively employed sulfur mustard against military and civilian targets and caused casualties in both populations. Some of the victims survived the attacks. This group faced different fates depending on the intensity and duration of initial exposure. Those who had been exposed minimally overcame the effects and are living a normal life, but many others developed serious problems–mainly pulmonary–with a progressive course. There are also a group of patients who have been asymptomatic or with minimal symptoms but whose conditions gradually deteriorated. The reasons remain to be elucidated.

Immunological mechanisms might be involved. The effects of nitrogen mustard on the immune system (immunosuppression, leukopenia) have been well studied. But not much is known about sulfur mustard. In this article the long-term complications of sulfur mustard on humoral immunity are investigated.

MATERIALS AND METHODS

Patients

179 individuals who were injured by mustard gas during the Iran-Iraq war (172 men and 7 women), were studied. The mean age was 33 years. They were injured between 1983-1988. Since this study was initially carried out in 1992, 3-8 years have elapsed between the date of injury and the date of the survey, averaging 6.1 years.

Since respiratory problems were the major complaint of these patients, they had been clinically divided into 3 groups according to clinical findings as well as spirometric, oximeteric and chest x-ray evaluations. Hence group 1 consisted of 81 patients (78 men and 3 women). There were 52 patients (50 men and 2 women) in group 2 and 46 patients (44 men and 2 women) in group 3.

Control group

49 healthy individuals (29 men and 20 women) with a mean age of 32 years were chosen. They have been randomly selected and were examined thoroughly before selection.

Assay

Serum IgG, IgA, C3 and C4 were measured using SRID. Serum IgE was measured by ELISA.

Statistics

INSTAT software was employed to carry out statistical calculation.

RESULTS

Humoral immunity parameters were measured in each group and compared to the control group.

IgG

IgG levels in all 3 groups were significantly lower as compared to the control group (Table I).

IgA

IgA levels in groups 1 (mean 2.80 ±1.24) and 2 (mean 2.87 ± 1.36) were not significantly different from controls (mean 2.81 ± 1.06). However, patients in group 3 had significantly lower IgA levels as compared to controls (mean 2.27 ± 1.07; $p = 0.012$).

IgE

IgE levels in groups 1 (mean 88.11), 2 (mean 60.50) and 3 (mean 123.0) were not significantly different from the control group (mean 116.58).

C3

C3 levels in group 1 and 2 (means 1.04 ±0.28 and 0.92 ± 0.28, respectively) were not significantly different from the control group (mean 1.05 ± 0.37). Again, patients in group 3 demonstrated significantly lower C3 levels (0.90 ± 0.29, $p = 0.004$). The same held true for C4 levels with mean values of 0.39 ± 0.15 and 0.38 ± 0.40 for groups 1 and 2, respectively, which were not significantly different from the control group (0.38 ± 0.11). Patients in group 3 had significantly lower C4 levels. The mean C4 value in the control group was 0.31 ± 0.16 ($p = 0.002$).

A significant decline in IgA, C3 and C4 levels was restricted to group 3. IgG levels had also decreased significantly in this group. It is noteworthy that 19.6% (9 out of 46) of patients in group 3 (mild) had lower than normal IgG levels. Lower than normal IgG levels were seen in 15.2% (7 out of 46) of group 3, 3.8% (3 out of 80) in group 1, 5.8% (3 out of 52) in group 2 and none of the control group subjects.

Four out of seven patients in group 3 with lower than normal IgA levels had concomitant low IgG levels. Two of these latter patients gradually developed more serious

Table I. Evaluation of humoral immunity in mustard gas victims.

Group	Control	Severe	Moderate	Mild
IgG	15.91± 2.29	14.47±4.87	14.63±4.40	13.07±5.56
IgA	2.81±1.06	2.80±1.24	2.87±1.36	2.27±1.07
IgE	116.58	88.11	60.50	123.0
C3	1.05±0.37	1.04±0.28	0.92±0.28	0.90±0.29
C4	0.38±0.11	0.39±0.15	0.38±0.40	0.31±0.16

respiratory problems and were categorized as severe patients 2 years later.

These results indicate that significant decreases in serum IgG, IgA, C3 and C4 levels occurred only in group 3. Also, those with concomitant low IgG and IgA levels are more prone to have a progressive course of lung disease and later develop more serious problems.

DISCUSSION

The mechanisms by which sulfur mustard induces lung or skin injuries has not been fully elucidated. DNA base substitution,[3] DNA alkylation,[4] increased protease activity,[5] decreased NAD[6] and released free radicals[7] are among the proposed mechanisms. In case of NAD, sulfur mustard activates poly ADP ribose polymerase. This will eventually lead to decreased intracellular NAD. Yourick reported that niacinamide (a poly ADP ribose polymerase inhibitor) can prevent blisters in mice exposed to mustard gas.[6]

The role of sulfur mustard in induction of malignancies has long been debated. Many authors have proved a relation with skin,[8] upper respiratory tract,[9] and nasal malignancies,[10] while others have observed no relation between sulfur mustard and lung cancer.[11]

The effects of sulfur mustard on the immune system has been less well studied. Lymphopenia has been reported in 535 mustard injured patients by Momeni et al.[12] Coutelier et al. demonstrated decreased B cells without functional defects in animal models.[13] Johnson and Meier showed mustard cytotoxicity on lymphocytes. Niacinamide and 3-aminobenzamide prevented this effect.[14] Islami and Keyhani observed decreased serum IgG levels in the first week after exposure in 120 patients (unpublished data). Elyasi and Deihimi also observed normal humoral immunity in one hundred patients one year after exposure (unpublished data). Vosoughi et al. reported defects in cellular immunity in these patients (unpublished data). We are reporting decreased IgA, IgG, C3 and C4 levels in patients in the mild group. The complement system consists of more than 30 fluid phase and cell membrane proteins that together play important roles in defence against infections and in regulation of inflammation. Complement provides a system capable of recognizing a large variety of pathogens and targeting them for destruction, either directly or by recruiting phagocytic cells. The primary site for biosynthesis of the majority of fluid phase components, including C3 and C4, is the hepatocyte. Inherited C3 and C4 deficiencies are known to be associated with increased bacterial infection,[15,16] systemic lupus erythematosus[17] and other autoimmune disorders.[18]

Non-inherited forms of C3 and C4 deficiencies, as seen in the mild group of our patients should be due to increased consumption of these components and not defects in their biosynthesis in the liver (liver function tests were normal in all groups of our patients). Hypocomplementemia is an important predisposing factor in some patients with recurrent pneumonia, a common condition in mustard injured patients.[19]

IgA plays an important role in mucosal immunity. Through it's receptors it can enter epithelial cells and pass through them and bind to the antigens present in the lumen and hence prevent their attachment and subsequent penetration to epithelial cells. During passage through epithelial cells, IgA can neutralize intracellular viral components. Also by attachment to antigen-antibody complexes formed in the submucusa, IgA can guide them through the cells into the lumen and hence prevent type III reactions.[20] Decreased IgA in mucosal surfaces which is usually accompanied by it's decreased serum level and concomitant serum IgG_2 levels can seriously impair mucosal defence and is associated with increased respiratory tract infections.[21] Bronchiectasia is also among the conditions associated with low IgA levels.[22] Recently it has been reported that concomitant low IgA and C4 levels can cause various immunological disorders.[23]

Sulfur mustard is not immunogenic by itself but can act as a hapten. In 1992 Liesek et al. were able to produce anti-sulfur mustard antibody after attaching the molecule to KHL.[24] This antibody is used for diagnostic purposes and it does not appear that this antibody is produced *in vivo*. After the body is exposed to this agent, it quickly enters the cell and after binding to DNA or via other mechanisms,

causes cell injury. Then it is rapidly metabolized and excreted. If the exposure rate is high, serious injuries will ensue in different organs (mainly the respiratory system), whose effects can be studied in the severe group of patients.

It is postulated that if the exposure rate is low, minor intracellular injuries can lead to antigenic structural changes of cellular proteins. These new antigens are not within accessibility of the immune system. Gradually with cell death, these antigens are introduced to the immune system and subsequently delayed mustard gas injuries will ensue in the mild group of patients. Some of these antigens may cause suppression of mucosal immune systems and decreased IgA levels by complicated, as yet unelucidated mechanisms.

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