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CELL-MEDIATED IMMUNITY AND IMMUNOGLOBULIN DETERMINATIONS IN PERSONS OCCUPATIONALLY EXPOSED TO BERYLLIUM

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ABSTRACT

Immunologic mechanisms were hypothesized to contribute to the pathogenesis of berylliosis and now these have been reasonably well documented. This work was undertaken to evaluate cell-mediated immunity and immunoglobulin levels of 30 healthy workers, 27 males and 3 females, between 21 and 49 years old, occupationally exposed to beryllium or its compounds at concentrations in air below Threshold Limit Values (TLV). The cellular immunity was measured by skin reactivity to 1% beryllium sulphate ($BeSO_4$) and 1% beryllium chloride ($BeCl_2$) according to the method of "Routing Patch Testing II", and *in vitro* specific (with $BeSO_4$) and nonspecific (with phytohaemagglutinin – PHA) blast transformation (BT) test of lymphocytes from the peripheral blood; the cultures were set up according to the modified method of Younge.

A delayed type of skin hypersensitivity to beryllium salts was shown in 26.6% of persons of the investigated group; these data are in a statistically significant correlation to exposure time to beryllium by Yates' χ^2test. No correlation was found between the results of specific patch tests and BT; this phenomenon may depend on different range of individual reactivity of T-lymphocytes to beryllium antigens. The increased serum IgA level was statistically significant in comparison to the control group. The additional studies should be carried out including the determination of specific antibodies to exclude nonspecific IgA elevation.

To summarize, some evidence of specific cell-mediated reactivity to beryllium has been ascertained in a number of healthy workers exposed to this metal at air concentrations below TLV.

Sterner and Eisenbud[23] were the first who clearly stressed an immunologic pathogenesis of chronic berylliosis. Many proofs have been gathered about the mechanisms involved in the disease[1,5,13]. The results of experimental investigations and study in persons suffering from chronic pulmonary berylliosis indicated that hypersensitivity of the cell-mediated type played an important role in the disorders[16,17,18,20]. This has been documented by the *in vitro* positive transformation of lymphocytes and inhibition of peripheral blood leucocyte migration in workers exposed to beryllium salts as well as by *in vivo* specific patch tests[8,9] in patients with diagnosed berylliosis. The passive transfer of sensitive lymphocytes and inhibition of skin reaction to beryllium by antilymphocyte serum in experiments confirmed the share of cellular hypersensitivity[3,4,7,17].

The correlation between changes of immunologic reactivity and onset of chronic berylliosis is not yet clear[15]. Therefore the investigations of immunologic reactivity in healthy persons exposed to beryllium are expected to reveal new details about this problem.

This study was undertaken to evaluate the cellular immunity in persons occupationally exposed to beryllium by examinations of the nonspecific and specific responsiveness of peripheral blood lymphocytes (PBL), the specific patch test, and the nonspecific humoral immunity by determinations of the circulating serum immunoglobulin levels IgG, IgA, IgM.

SUBJECTS, MATERIAL AND METHODS

The investigated group consisted of 30 healthy workers (27 males, 3 females, aged 21 – 49) employed in mechanical, thermal or chemical handling of 2% beryllium alloys in the environment contaminated with beryllium below TLV. The time of exposure to beryllium ranged from 4 to 72 months. The examined group was divided into the following three subgroups according to the period of employment: 1) time of exposure up to 6 months, 2) time of exposure 6 to 24 months, 3) time of exposure over 24 months.

The control group consisted of 30 persons (25 males, 5 females, aged 21 – 49) who had no contact with beryllium.

Patch tests with 1% beryllium sulphate ($BeSO_4$) and 1% beryllium chloride ($BeCl_2$) were made according to the method proposed by Routing Patch Testing II[20]. An estimation of the results was based on recommendations of WHO[25].

In vitro blast transformation with beryllium sulphate and phytohaemagglutinin

Cultures of PBL were prepared according to the method of Ling[19]. The medium for each culture consisted of 3 ml Parker's solution produced by Biomed, containing 10 μg/ml of kanamycin. To this medium 3 ml of autologic plasma and 0.1 ml of suspension of investigated lymphocytes were added. For each subject 12 tissues cultures were prepared. Beryllium sulphate ($BeSO_4$) was added to six tubes: 7 μg/ml to the first three tubes and 0.7 μg/ml to the other three. The amount of 50 μl of phytohaemagglutinin (PHA M-difco, provided by Welcome) was added to the next three tubes. The last three tubes – without PHA or $BeSO_4$ – served as control. The cultures containing PHA and one control culture were incubated for 72 hours at 37 °C and the remaining cultures for 144 hours. Cell suspension was then centrifuged, cells were washed with Hanks balanced solution (HBS), fixed in a cold mixture of glacial acetic acid (1 volume) and methanol (3 volumes) and stained with May-Gruenwald-Giemsa stain. Percentage blast transformation was determined by morphologic count of 1 000 lymphocytes per slide. The results indicated the ratio of blast transformed (BL) cells to total number of leucocytes. The differences between the levels of BL of PHA or $BeSO_4$ stimulated cultures and the control culture were compared.

Circulating serum immunoglobulin levels IgA, IgG, IgM were determined by the agar immunodiffusion technique according to the modified method of Manci ini[12], with the standard reagent provided by Behring.

RESULTS

The results of the specific and nonspecific blast transformation (BT) are summarized in Table 1. The specific stimulation of lymphocytes showed the increased level of BT in one worker only, while the impaired PHA response was observed in two workers. The responsiveness of lymphocytes in other workers

TABLE 1

Response of peripheral blood lymphocytes (PBL) to phytohaemagglutinin (PHA) and beryllium sulphate ($BeSO_4$). Ratio of blast transformed (BL) cells to total number of leucocytes.

		Workers exposed to beryllium (months)			Control group	F ($r_1 = 35$) ($r_2 = 3$)
		0–6 (n = 7)	6–24 (n = 11)	over 24 (n = 11)		
A	$\bar{X}$	0.24	0.74	0.98	0.54	0.79
	S.D.	±0.48	±0.96	±1.25	±0.91	
B	$\bar{X}$	67.42	67.72	65.68	64.99	0.16
	S.D.	±7.25	±6.40	±15.33	±8.81	
C	$\bar{X}$	0.37	0.97	2.00	1.02	1.44
	S.D.	±0.32	±0.69	±3.44	±0.88	
D	$\bar{X}$	0.31	0.91	2.08	0.72	1.55
	S.D.	±0.29	±0.67	±3.38	±0.84	

A = results of PBL blast transformation in control cultures

V = results of PBL blast transformation in PHA nonspecific stimulated cultures

C = results of PBL transformation in $BeSO_4$ specific stimulated cultures

D = results of differences between blast transformation level in specific stimulated ($BeSO_4$) and control cultures

was found to be within normal range. There were no significant differences in results between the exposed and control workers either in specific or nonspecific PBL-response; nevertheless a tendency to the increase of specific blast transformation was observed in subgroup III, the subjects exposed to beryllium for over 24 months.

Specific patch test was positive in 8 subjects out of 30 : 3 positive reactions were observed in subgroup II and 5 in subgroup III. The results of skin tests

analyzed by Yates' X^2 tests, as presented in Table 2, indicated that the frequency of positive tests depended on the time of exposure to beryllium.

TABLE 2
The analysis of results of skin tests according to the time of exposure to beryllium by Yates' χ^2 tests.

Time of exposure	Negative	Positive	Total
0–6 months	17	0	17
Over 6 months	15	8	23
Total	32	8	40

$\chi^2 = 7.39$; $p < 0.01$

The results of serum immunoglobulin level determinations are shown in Table 3. The coefficient of correlation between the results of investigated and control groups was highly significant ($p < 0.01$, t-test) with reference to IgA level, while the IgG and IgM were not statistically significant ($p > 0.05$, t-test).

TABLE 3
Results of serum immunoglobulin determinations in workers exposed to beryllium and in control group by Snedecor's test.

Immuno-globulin (I.U.)	Control group (n = 30)	Workers exposed to beryllium			Total (n = 30)	F
		0–6 months (n = 7)	6–24 months (n = 11)	over 24 months (n = 12)		
IgG	147.06 ± 47.53	141.42 ± 31.18	164.54 ± 26.21	163.33 ± 46.38	158.66 ± 36.71	0.86*
IgA	132.30 ± 40.77	167.14 ± 53.84	237.27 ± 101.77	201.25 ± 97.45	206.50 ± 92.29	7.10***
IgM	144.33 ± 47.59	111.57 ± 37.93	110.00 ± 45.33	126.91 ± 36.69	118.46 ± 40.38	2.21**

*below the level of significance: $p > 0.05$
**not significant: $p > 0.1$
***highly significant: $p < 0.01$

The results of immunologic determinations in persons with positive patch tests' with beryllium sulphate and beryllium chloride are shown in Table 4.

TABLE 4
The results of immunologic determinations in eight persons with positive patch tests with beryllium sulphate ($BeSO_4$) and beryllium chloride ($BeCl_4$).

Specific patch tests	Response (%) of peripheral blood lymphocytes to:		Immunoglobulin (I.U.)		
	PHA	$BeSO_4$	IgG	IgA	IgM
+++	N	N	N	N	N
+++	N	N	N	–	N
+++	N	12.0	N	N	N
+++	N	–	N	N	N
++	N	N	N	320	N
++	N	N	N	N	N
++	48.0	N	N	N	N
++	N	N	N	N	N

N = within normal range

DISCUSSION

The occurrence of cell–mediated immunity in persons suffering from chronic pulmonary berylliosis has been well documented[14,21], while in the healthy population in occupational contact with beryllium its evidence has not been so obvious. Our findings of positive specific skin tests in 26 per cent of investigated subjects proved that among apparently healthy workers working under good environmental conditions a delayed skin hypersensitivity to beryllium may occur. The specific patch test may support the diagnosis of the disease as emphasized by several authors[10]. In persons with no other manifestations of the disorders positive skin reaction to beryllium salts may indicate the "cellular memory" or even "cutaneous memory" to the provocative factor only.

PHA and $BeSO_4$ were used as stimulators of nonspecific and specific responsiveness of lymphocytes, respectively. The results of PBL specific blast transformation tests appeared nonsignificant compared with the control ($p > 0.05$, F-test). It seems that prolonged exposure to beryllium may lead towards the increase of sensitivity of PBL resulting in a slight tendency to higher specific transformation (subgroup III - Table 1). The significantly increased PBL response to $BeSO_4$ was found in one case only. It is interesting that this person showed a positive reaction in specific patch test as well, but X-ray examinations and functional pulmonary test indicated no changes. The subject was estimated as a person at higher risk of the disease and was withdrawn from contact with beryllium. There are two possible explanations of the phenomenon: either that the person is to reveal the disease in the future or the individual immunological reactivity is of a wide range and needs additional factors to stimulate the onset of chronic berylliosis[2]. The results of specific patch test and PBL blast transformation tests were not clearly correlated. One can suppose that these two

tests may examine the different functions of T-lymphocytes connected for instance with various lymphokinin – releasing factors. According to the data presented by Deodhar and co-workers[11] the incidence of PBL reactivity to beryllium salts among industrial workers was small, only two cases out of 30 exposed workers and two out of 22 normal healthy subjects. Vasil'eva and co-workers[24] found cell-mediated immunity against beryllium salts in 36 per cent of workers exposed to beryllium, in 7 per cent when measured by migration inhibition tests (MIT), and in 3 per cent when examined by patch test.

It has been found recently that humoral reactivity is involved in the pathogenesis of berylliosis especially in the interstitial form of the disease. Our findings prove that serum IgA of the investigated group was significantly higher than in the control ($p < 0.01$, t-test). The results of the determinations of immunoglobulin levels either in patients with diagnosed chronic berylliosis or in beryllium industry workers greatly differ. Deodhar and co-workers[11] observed increased IgA level in patients with chronic pulmonary berylliosis, while Resnick and co-workers[22] pointed out significant IgA elevations. Vasil'eva and co-workers[24] found an increase in both IgA and IgG levels in patients with berylliosis and in beryllium industry workers.

The higher level of IgA in our group of exposed workers may be due to the effect of beryllium on the respiratory membrane. As IgA elevation accompanies several diseases and may occur among normal subjects[6], the specific antibodies should be determined before drawing final conclusions.

The investigations of immunologic responsiveness are valuable because they may reveal early changes caused by occupational agents such as beryllium, especially in the subjects who have not developed clinical symptoms.

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