IMMUNOGLOBULIN CONCENTRATIONS IN BERYLLIOSIS[1]

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SUMMARY

The relative concentrations of the constituent immunoglobulins in the serum of subjects with berylliosis were determined. Included in the group were subjects with resolved acute berylliosis, patients with the chronic form of the disease, and a few subjects who had had beryllium dermatitis. Most subjects who had had either the cutaneous or the chronic pulmonary forms of the disease manifested hypergammaglobulinemia and an increased concentration of IgG. This abnormality was less frequent in subjects with resolved acute berylliosis. A significantly increased concentration of IgG was found in several workers who had had long exposure to either the metal or its compounds but who had never had any evidence of beryllium toxicity.

INTRODUCTION

The diagnosis of chronic berylliosis is difficult because it depends almost entirely on circumstantial evidence. The abnormalities of pulmonary function found in the disease are nonspecific, and its close resemblance to sarcoidosis clinically, physiologically, and pathologically poses a recurring problem. A further difficulty is that only a small minority of workers who are exposed to beryllium contract the disease, and there is much evidence to suggest that hypersensitivity is responsible in those who do (1). This possibility is supported by the observation that berylliosis is often accompanied by hypergammaglobulinemia (2), and it seems reasonable to infer that the mechanisms responsible for the increase in gammaglobulin might be intimately concerned in the pathogenesis of the disease. The present study, therefore, was undertaken to determine the relative concentrations of the constituent immunoglobulin fractions. The serum concentrations of IgG, IgM, and IgA were determined in a group of subjects who demonstrated one of the several manifestations of berylliosis.

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MATERIALS AND METHODS

With one exception, all serum specimens tested in this study came from former or current employees of a beryllium refinery. The exception was a patient with proved chronic berylliosis who, since the disease was diagnosed in 1962, had been followed as an outpatient at the West Virginia University Medical Center. All the subjects were white men except *Subject 6* in group 1 who was a white woman.

Serum samples: Serum was obtained from 5 groups of subjects. Group 1 consisted of 6 subjects with proved chronic berylliosis, all of whom met the criteria for inclusion in the Beryllium Register; group 2 consisted of 7 subjects who had had acute berylliosis that had resolved without apparent residual symptoms; group 3 consisted of 5 subjects who had had dermatitis sometime during their employment but who were currently free of the condition; group 4 consisted of 22 subjects who had been employed in the beryllium plant for 15 years or more but who had never had any of the known manifestations of berylliosis; and group 5 consisted of 3 subjects who had had symptoms suggestive of acute or chronic pulmonary disease but who eventually were proved to have had a nonoccupational respiratory disease. All serum specimens were stored at −20°C until ready for use.

As a check on the immunodiffusion technique and to verify the normal values for IgG, IgM, and IgA, 20 specimens of serum were drawn from normal hospital employees and blood donors. These specimens were also used as controls.

Electrophoretic analyses: The distribution of individual serum proteins was determined by cellulose acetate strip electrophoresis. The specimens were electrophoresed in Tris-barbital buffer, pH 8.8, I = 0.05, on 1-inch by 6.75-inch Sepraphore

TABLE 1

IMMUNOGLOBULIN CONCENTRATIONS IN SUBJECTS WITH PROVED CHRONIC BERYLLIOSIS

Subject	Age (yrs.)	Gamma globulin (% of total protein) Normal Range: 11 to 15	IgG (mg/100 ml) Normal: 1,263 ± 395	IgM (mg/100 ml) Normal: 114 ± 58	IgA (mg/100 ml) Normal: 240 ± 91	Radiologic Findings	Lung Biopsy	Patch Test	Dyspnea	Year of Onset	Receiving Corticosteroids
1	54	25.7	3,120	78	437	Perihilar shadows and diffuse reticulonodulation	Granulomas and fibrosis	Not done	3+	1965	Yes
2	35	23.7	2,420	245	715	Enlarged hilar nodes and diffuse reticulation	None	Reactive	2+	1959	Yes
3	47	14.2	1,450	220	365	Enlarged hilar nodes and diffuse reticulonodulation	Granulomatous reaction and interstitial fibrosis	Not done	3+	1961	Yes
4	34	18.2	2,420	78	178	Perihilar infiltrate and reticulonodulation	Granulomatous reaction and interstitial fibrosis	Not done	3+	1964	Yes
5	53	18.7	2,420	131	254	Perihilar infiltrate	Granulomas throughout lungs	Not done	3+	1961	Yes
6	51	18.5	1,820	122	170	Diffuse reticulonodulation	Interstitial fibrosis and granulomas	Not done	3+	1962	Yes

III cellulose acetate strips[2, 3]; the milliamperage per strip was approximately 1.0 ma to 1.2 ma. The separated fractions were stained with ponceau S; densitometric recording and quantitation of the electrophoretic patterns were made with a Densicord Model 542 equipped with an integrator.[4]

Immunodiffusion: The concentrations of immunoglobulin fractions (IgA, IgG, and IgM) were estimated using commercially available immunoplates test kits,[5] which contained the antiserum specific for each fraction: IgA, Lot No. 7012EO, 16Cl; IgG, Lot No. 7002EO 10Bl; IgM, Lot No. 7022EO 11Al. The manufacturer's suggested normal values were as follows: IgG, 1,200 ± 300 mg per 100 ml; IgM, 80 ± 29 mg per 100 ml; IgA, 288 ± 121 mg per 100 ml. Because there was considerable variation in the "normal" values from one batch of immunodiffusion plates to that of another, the 20 serum samples obtained from normal donors were examined for their immunoglobulin concentrations. The mean ±SD and the range of values were as follows: IgG, 1,263 ± 395 mg per 100 ml (868 to 1,658 mg per 100 ml); IgA, 240 ± 91 mg per 100 ml (149 to 331 mg per 100 ml); and IgM 114 ± 58 mg per 100 ml (56 to 172 mg per 100 ml). These values were in close agreement with those reported by other investigators (3, 4) and were used as a basis for comparison. They are listed in the various tables.

[2] Mention of commercial products or concerns does not constitute endorsement by the U. S. Department of Health, Education, and Welfare.

[3] Gelman Instrument Company, Ann Arbor, Michigan.

[4] Photovolt Corporation, New York, New York.

[5] Hyland Laboratories, Los Angeles, California.

RESULTS

In group 1 (table 1), 4 of the 6 patients with proved chronic berylliosis showed IgG concentrations greater than 2 SD above the mean; one of the 6 had an IgG concentration greater than 1 SD above the mean. On the other hand, the increased concentrations in the other immunoglobulin constituents did not follow any particular pattern. For example, *Subjects 1* and *2* had an increased IgA concentration (more than 2 SD above the mean) whereas in *Subject 3* the increase was more than 1 SD. The IgM concentrations also were increased for *Subjects 2* and *3*. The general increase in immunoglobulins in *Subject 2* suggested the possibility that undefined fac-

TABLE 2

IMMUNOGLOBULIN CONCENTRATIONS IN SUBJECTS WITH ACUTE BERYLLIOSIS (RESOLVED)

Subject	Age (*yr*)	Gamma globulin (*% of total protein*)	IgG (*mg/100 ml*)	IgM (*mg/100 ml*)	IgA (*mg/100 ml*)
1	45	21.8	2,750	131	305
2	55	17.1	1,450	178	285
3	28	16.9	2,750	107	525
4	46	13.2	1,390	64.5	400
5	38	13.9	2,370	89	286
6	57	14.9	2,370	258	550
7	56	10.1	1,200	24.5	95
Normal		11–15	1,263 ± 395*	114 ± 58*	240 ± 91*

* mean ± SD

TABLE 3

IMMUNOGLOBULIN CONCENTRATIONS IN SUBJECTS WITH DERMATITIS

Subject	Age (*yr*)	Gamma globulin (*% of total protein*)	IgG (*mg/100 ml*)	IgM (*mg/100 ml*)	IgA (*mg/100 ml*)
1	50	16.7	2,070	98.5	160
2	46	13.2	2,070	71.5	286
3	40	9.4	1,200	42	160
4	49	17.9	2,370	58	350
5	58	15.4	1,820	24.5	286
Normal		11–15	1,263 ± 395*	114 ± 58*	240 ± 91*

* mean ± SD

tors other than berylliosis might have been operative. In *Subjects 4, 5,* and *6,* only the serum IgG was appreciably increased. It should also be pointed out that all the patients in group 1 were taking corticosteroids at the time the serum samples were drawn.

In the subjects who had had acute berylliosis, 4 of the 7 had increases of serum IgG with values greater than 2 SD above the mean (table 2). Once again, the concentrations of the other immunoglobulin constituents (IgA and IgM) varied, and did not follow any particular pattern. In some instances (*Subject 6*), there was a concomitant increase in the other two immunoglobulins whereas in others (*Subjects 2, 3,* and *4*) only one fraction was increased. There was apparently no obvious relationship between the concentrations of IgG and the concentrations of other immunoglobulin constituents in these subjects.

In group 3 (table 3), 4 of the 5 subjects in whom dermatitis developed while they were working at the plant also demonstrated increased IgG concentrations; 3 of these had concentrations that exceeded the mean by more than 2 SD. All patients in this group showed normal IgA and IgM concentrations with the exception of *Subject 4,* who showed an IgA concentration greater than 1 SD above the mean. Although these patients had dermatitis, the clinical picture was sometimes nonspecific, and it should be stressed that the association between the skin lesions and occupation was occasionally tenuous.

Group 4 (table 4) subjects had a history of long exposure to the metal but had never demonstrated any of the manifestations of toxicity. In this group, 13 of the 22 subjects demonstrated marked increases of IgG, i.e., values exceeding the mean by more than 2 SD, whereas 5 of the subjects showed increases greater than 1 SD; this represented more than 80 per cent of the subjects with an increased IgG concentration. The concentrations of the other immunoglobulin fractions varied from one patient to another; 7 of the 22 showed concentrations of IgA more than 2 SD greater than

TABLE 4

IMMUNOGLOBULIN CONCENTRATIONS IN SUBJECTS WITH LONG EXPOSURE BUT NO BERYLLIOSIS

Subject	Age (*yr*)	Gamma globulin (*% of total protein*)	IgG (*mg/100 ml*)	IgM (*mg/100 ml*)	IgA (*mg/100 ml*)
1	42	12.2	1,200	122	485
2	50	16.8	2,070	169	286
3	62	17.0	1,820	110	485
4	61	17.4	2,710	187	350
5	50	9.4	1,390	89	150
6	45	13.2	1,820	52	590
7	49	14.6	2,070	110	250
8	53	18.1	2,070	64.5	140
9	64	19.6	2,710	71.5	400
10	49	13.6	2,070	152	236
11	57	15.9	2,070	89	485
12	40	17.0	2,370	122	270
13	41	16.0	1,590	137	375
14	43	19.2	2,070	152	306
15	42	29.1	3,780	80	485
16	49	13.1	1,590	98.5	250
17	55	15.4	2,070	89	350
18	56	13.4	1,820	152	236
19	52	17.2	1,820	64.5	425
20	53	17.5	2,370	71.5	328
21	53	14.2	1,820	71.5	306
22	54	14.8	2,070	46.5	485
Normal		11–15	1,263 ± 395*	114 ± 58*	240 ± 91*

* mean ± SD

the mean whereas 3 had concentrations greater than 1 SD above the mean. Only one patient (*Subject 4*) showed an increase of IgM greater than 1 SD.

Group 5 (table 5) consisted of 3 subjects who at one time had pulmonary symptoms suggestive of berylliosis but in whom the disease was not confirmed. In this group, only *Subject 24* showed an IgM concentration more than 2 SD greater than the mean; the other immunoglobulin concentrations were well within the normal limits.

The data indicated that most patients with long exposure to beryllium had increases of serum IgG, a finding that was also present in a large number of subjects with resolved acute berylliosis and dermatitis.

DISCUSSION

Although the occupational hazards of beryllium extraction have been recognized since the 1940's, sporadic cases of acute and chronic berylliosis still occur. The diagnosis of chronic berylliosis is fraught with difficulty and depends usually on circumstantial evidence alone. For instance, the granulomatous changes found in the lymph nodes and lungs of subjects with berylliosis are nonspecific and cannot be differentiated from those found in sarcoidosis (2). To complicate the issue further, a history of exposure to beryllium or its compounds plus the nonspecific clinical features of a diffusion fibrosis by no means excludes sarcoidosis because sarcoidosis occasionally occurs in beryllium workers. In chronic berylliosis, on the other hand, although a history of occupational exposure to the metal is usual, it is not essential to the diagnosis. Conversely, the demonstration of an excess of beryllium in the body indicates exposure to the metal sometime in the subject's life, but it cannot be construed as evidence for the presence of the condition. The metal remains in the tissues for many years and is very slowly excreted; moreover, the amount of beryllium detected in the tissues bears no relation to the severity of the disease. The difficulties in establishing the diagnosis are further compounded by the fact that berylliosis develops in only a very small minority of those exposed to the metal and its com-

TABLE 5

IMMUNOGLOBULIN CONCENTRATIONS IN SUBJECTS WITH PULMONARY SYMPTOMS SUBSEQUENTLY SHOWN NOT TO HAVE BERYLLIOSIS

Subject	Age (*yr*)	Gamma globulin (*% of total protein*)	IgG (*mg/100 ml*)	IgM (*mg/100 ml*)	IgA (*mg/100 ml*)
23	47	15.2	1,650	87	286
24	41	13.6	1,280	410	240
25	46	16.5	1,650	118	163
Normal		11–15	1,263 ± 395*	114 ± 58*	240 ± 91*

* mean ± SD

pounds. It has been suggested, therefore, that the pathogenesis of berylliosis is related to hypersensitivity (1). In support of this possibility is the hypergammaglobulinemia found in the condition and the exacerbations of the disease that can result from patch testing (5). Perhaps the two most useful methods of differentiating sarcoidosis from berylliosis are the Kveim-Nickerson and beryllium patch tests. Neither is entirely satisfactory because the Kveim test depends on obtaining a suitable active antigen, and this often proves very difficult. With regard to the patch test, lack of a reaction does not exclude the diagnosis of berylliosis (6). Thus, some other diagnostic test for the condition seems to be needed.

IgG immunoglobulin normally constitutes 15 per cent to 25 per cent of the total human serum protein. Hypergammaglobulinemia occurs commonly in chronic granulomatous infections of long duration, with the increase in IgG representing both specific antibodies and nonspecific IgG immunoglobulins. Hypergammaglobulinemia is also found in diseases associated with hypersensitivity granulomas and in a variety of dermatologic conditions related to hypersensitivity (4). Although hypergammaglobulinemia has long been known in berylliosis the writers could not find reports of studies of the individual immunoglobulins in this condition. The presently reported finding of a fairly consistent increase in IgG concentration in chronic berylliosis might be of considerable significance and is in accord with the hypothesis that the disease is related to hypersensitivity. The change in IgG concentration also seems useful in differentiating berylliosis from sarcoidosis because according to Piotrowski and Wolanska (7), it is the IgA concentration that is most affected in the latter disease. A few subjects with berylliosis had minor to moderate increases of other immunoglobulins; these occurred at random and did not appear to have any obvious correlation with other factors.

The effect of corticosteroids on the production of immunoglobulins is uncertain in man, but in animals these compounds have been shown to reduce immunoglobulin concentrations (8). It is noteworthy that even though all subjects with the chronic form of the disease were taking corticosteroids, all but one had increases of IgG. It is tempting to surmise what might happen if their medication was discontinued for a period, but because all were symptomatic, and because all claimed they were helped by the drug, it was decided that such an intervention was unjustifiable.

The significance of the finding that 4 of the 7 subjects who at one time had acute berylliosis had increases of IgG concentrations is difficult to interpret, because the relationship of the acute and chronic forms of pulmonary berylliosis is obscure. In most of the subjects in whom acute berylliosis developed the disease apparently regressed completely, but the possibility exists that with continued exposure, in some the acute process slowly evolves into the chronic form. An analogous situation exists in farmer's lung, and because both diseases probably have their origin in hypersensitivity, there is a fair amount of circumstantial evidence to support this hypothesis (9). It is possible that those subjects who show a persistent increase of IgG after the acute attack are those in whom the chronic form of the disease is likely to develop. The same hypothesis may be applicable to the group who

have had dermatitis because virtually all of them also had significant increases of serum IgG.

Most interesting of all is the group of subjects who had no history of acute, chronic, or cutaneous berylliosis. All had worked in close contact with the metal or its compounds for many years, but other than *Subjects 23, 24,* and *25* (group 5), none had had any symptoms to suggest berylliosis. Nonetheless, several subjects demonstrated marked IgG increases (more than 80 per cent of the 22 subjects in table 4; 13 with concentrations greater than 2 SD above the mean). There are two possible explanations for this finding; viz., these subjects are hypersensitive and, therefore, chronic berylliosis is more likely to develop in them. This is an attractive theory, but there is little evidence to support it (2). In favor of it is the fact that they apparently had no other disease process to account for their hypergammaglobulinemia and increased IgG concentrations nor anything else in common except exposure to beryllium. Although beryllium has been shown to resemble Freund adjuvant in its ability to potentiate antibody response, it lacks the facility to induce delayed hypersensitivity to incorporated protein antigens (10). It shares with Freund adjuvant the property of inducing a prolonged and heightened antibody response, and it is therefore possible that the increased concentration of IgG was a consequence of a previous antigenic challenge, and that the persistence of the elevation can be attributed to continuing exposure to beryllium.

Long term studies are obviously necessary to decide which of these two hypotheses is correct. If the increased IgG concentration were shown to be an indicator of the hypersensitive state, it could be used to identify those subjects who are susceptible to chronic berylliosis.

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RESUMEN

NIVELES DE INMUNOGLOBULINA EN LA BERILIOSIS

Se determinaron las concentraciones relativas de las inmunoglobulinas en el suero de sujetos con beriliosis. Se incluyeron en la serie, sujetos con beriliosis aguda resuelta, con la forma crónica de la enfermedad o con antecedentes de dermatitis por berilio. En sujetos con la forma cutánea o pulmonar crónica de la enfermedad, se demostró hipergammaglobulinemia con un aumento en la fracción IgG. Este hallazgo fue menos común en sujetos con beriliosis aguda resuelta. Varios obreros presentaban una concentración de IgG notablemente aumentada, aunque jamás habían revelado evidencia de intoxicación con berilio, a pesar de una prolongada exposición al metal o a sus compuestos. Se discuten posibles mecanismos de este hallazgo.

RESUME

TAUX D'IMMUNOGLOBULINES DANS LA BERYLLIOSE

On a procédé à la détermination des concentrations relatives des fractions d'immunoglobulines dans des échantillons de sérum provenant d'individus souffrant de berylliose. Le groupe étudié comprenait des sujets avec une berylliose aiguë en résolution, des sujets présentant la forme chronique de la maladie, ainsi que certains individus ayant des antécédents de dermatite au beryllium. On a mis en évidence une augmentation de la concentration en IgG chez la plupart des sujets qui avaient souffert, soit de la forme cutanée de l'affection, soit de sa forme pulmonaire chronique. Une telle augmentation a été trouvée moins fréquemment chez des individus présentant une berylliose aiguë en résolution. On a également observé une augmentation significative de la concentration en IgG chez plusieurs travailleurs qui n'avaient jamais présenté aucun signe d'intoxication par le beryllium, mais qui avaient été néanmoins exposés pendant une longue période au beryllium métal ou à ses composés. On discute des mécanismes qui pourraient expliquer cette dernière observation.

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