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Comparison of lymphocyte transformation and macrophage migration inhibition tests in the detection of beryllium hypersensitivity

WR WILLIAMS, W JONES WILLIAMS

From the Asthma Research Unit, Sully Hospital, Penarth and the Welsh National School of Medicine, Department of Pathology, Llandough Hospital, Penarth

SUMMARY In seven patients with chronic beryllium disease (Be) the Be lymphocyte transformation test was positive in 100%, independent of steroid therapy, and was reproducible. The Be macrophage migration inhibition test was only positive in four of seven patients (57%) not on steroids, and was not reproducible. In 72 potentially exposed healthy beryllium workers the lymphocyte transformation test was negative in all subjects. The macrophage test was positive in four of 78 and again the results were not reproducible. The workers with positive results showed no differences in age, type or duration of employment from those with negative results and showed no evidence of disease. In addition, the macrophage test was positive in two of 45 non-exposed control subjects.

We also confirmed the above advantages of the lymphocyte transformation technique by using tuberculin antigen (PPD). The PPD lymphocyte transformation test gave positive results in approximately 60% of healthy beryllium workers, but the PPD macrophage test was only positive in 7%.

We conclude that the Be lymphocyte transformation test is the most sensitive and reproducible and advocate its use in the diagnosis of disease and in monitoring the health of potentially exposed workers.

Delayed-type hypersensitivity reactions may be measured in vitro using lymphocyte cultures.[1] These tests are particularly relevant to beryllium disease as they avoid skin testing with an extremely potent sensitiser. Hypersensitivity is an important feature of chronic beryllium disease, and although not diagnostic, is of value in diagnosis.[2] Other applications of in vitro beryllium hypersensitivity tests include:

(*a*) screening tests to detect the development of sensitivity in potentially exposed workers, who may be at increased risk of developing disease;

(*b*) as an indicator of industrial hygiene standard.

Two in vitro tests, macrophage migration inhibition and lymphocyte transformation, have been reported to be of value for detecting beryllium hypersensitivity (Table 1), although influenced to different degrees by steroid therapy. Previously, a survey of a beryllium metal factory using the Be macrophage migration inhibition test[6] showed that 14% of workers were sensitised. However, the test was positive in only one of six patients.

In the present survey we have compared the sensitivity and reproducibility of the two tests in a reinvestigation of the original factory, workers from a ceramic factory and patients with chronic beryllium disease. We also assessed the reliability of the two tests by the use of tuberculin antigen in the factory workers. In addition, the beryllium and tuberculin migration inhibition tests were performed on control subjects and patients with tuberculosis.

Table 1 *Previous reports of "in vitro" beryllium hypersensitivity tests*

Macrophage migration inhibition	*Lymphocyte transformation*
Jones Williams *et al*[3]	Hanifin *et al*[8]
2/7 patients positive (on steroids)	7 patients all positive
Henderson *et al*[4]	Deodhar[9]
3 patients positive	25/35 patients positive (on steroids)
Marx and Burrell[5]	Nishikawa *et al*[10]
6/7 patients positive (on steroids)	4 patients all positive (not on steroids)
Price *et al*[6]	Preuss[11]
50 workers 14% positive	571 workers: 36% weak positives —not persistent
1/6 patients positive (other patients on steroids)	37/47 patients positive—strong and persistent (on steroids)

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Material and methods

Details of the Be macrophage migration inhibition[6] and Be lymphocyte transformation tests[7] have previously been described.

Beryllium sulphate ($BeSO_4$) was used at 10^{-6} – 10^{-7} *M* and tuberculin (PPD; MAFF, Weybridge) at concentrations of 20 μg/ml (MIF test) and 7·5 μg/ml (LT test).

A MIF index

$$\frac{\text{(area of migration with antigen)}}{\text{(area of migration without antigen)}} \text{ of } < 0{\cdot}8$$

was taken as a positive value. In LT tests, a stimulation index (SI) =

$$\frac{\text{(cpm in cultures with antigen)}}{\text{(cpm in cultures without antigen)}} > 2{\cdot}0$$

was taken as a positive test.

Various modifications to our standard MIF test, based on the technique of Rocklin[12] were made. A more pure lymphocyte population was obtained by preincubating Ficoll-Hypaque separated mononuclear cells for two hours in MEM with 5% human serum, in plastic Petri dishes. Most monocytes were removed by adherence.

Lymphocyte supernatants (lymphokines) were concentrated (× 3 – × 39) as follows. Lymphocytes were cultured for one to three days in flat-bottomed plastic tubes with or without PPD (5 μg/ml). The medium was changed daily, pooled and control medium reconstituted with PPD prior to filtration (pore size < 0·2 μm) and dialysed. The contents of dialysis bags were evaporated to dryness with an air fan. These lymphokine preparations were reconstituted in MEM with 15% complement inactivated guinea pig serum and contained 15-105 μg/ml PPD.

SUBJECTS

Beryllium antigen

Beryllium patients The two tests were performed on seven patients, fulfilling most of the criteria for diagnosis.[2] All were men working with beryllium metal, alloys, or both. The predominant symptoms included, to varying degrees, lethargy, dyspnoea, cough and weight loss. All showed radiological evidence of pulmonary fibrosis. Kveim tests were made to assist in excluding sarcoidosis. Positive biopsies showed epithelioid cell granulomas (Table 2).

Beryllium workers The in vitro tests were compared in 72 factory workers. Factory No 1: 35 workers, all engaged in machining pure beryllium, including 30 previously tested.[6] Factory No 2: a total of 37 workers involved in ceramic manufacture using beryllium oxide.

Controls The macrophage test was further assessed in control subjects. These included 35 hospitalised male patients, who were tested before or 10 days after undergoing surgery (for example, piles, varicose veins, hernias, etc). Ten were patients with tuberculosis. They were all within the working population age group, and none had any known exposure to beryllium.

Tuberculin antigen

The macrophage test was also assessed in the same factory population, controls and tuberculosis patients. The lymphokine concentration modification of the macrophage test was carried out on 12 additional patients with tuberculosis and seven tuberculin-positive normal subjects.

Results

BERYLLIUM ANTIGEN

Chronic beryllium patients (Table 3)

The macrophage test was positive in four of seven (57%) and the transformation test was positive in all seven (100%). The transformation results were unaffected by steroid treatment, but in two of the six patients on steroids the migration test was negative.

Of three patients previously reported as macrophage migration-positive[3 6] only one was positive on this occasion.

Table 2 *Details of chronic beryllium patients*

Patient	*Age (yr)*	*Length of employment (yr)*	*Kveim test*	*Mantoux test*	*Be patch test*	*Be urine/tissues*	*Biopsy*
1	47	10	–	ND	+	ND	+
2	34	13	–	ND	+	–/–	–
3	59	20	–	–	–	–/ND	ND
4	37	7	ND	ND	ND	–/ND	ND
5	48	4	–	–	+	–/+	+
6	43	18	–	–	+	–/ND	+
7	54	3	–	ND	–	ND	+

ND = Not done.

Table 3 *Comparison of Be MIF and Be LT tests on patients with chronic beryllium disease*

Patients	*Be MIF ratio*	*Be LT (20% serum)*	
		CPM with Be	*SI*
1*	0·78+	5496	2·9+
2*	0·78+	11070	3·7+
3*	0·72+	7850	10·0+
4*	0·65+	5700	7·6+
5*	0·86−	36557	17·4+
6*	0·98−	14620	9·3+
7	0·93−	13871	22·3+
Positive rate	4/7		7/7

*on steroids; + = positive; − = negative; CPM = counts per minute; SI = stimulation index.

Healthy beryllium workers (Table 4)
The macrophage test was initially positive in 4 of 72 (5%) tested. Two of the four were available for retesting and one gave a negative result.

We were able to compare the results of macrophage tests in four workers previously reported as positive, Be MIF ratio <0·87.[6] Three were initially positive but on repeat testing all were negative. Four workers not examined in the 1977 survey had MIF ratios <0·87, but only two were positive at <0·8 (included in Table 4).

In all 72 workers, the lymphocyte transformation results were negative.

Control population
The macrophage test was positive in two of 45 (4%) subjects with no known beryllium exposure.

TUBERCULIN ANTIGEN
The PPD lymphocyte transformation test (Table 4) was positive in 58% of the healthy factory population and in all seven patients with chronic beryllium disease. The PPD macrophage test, however, showed a very low positive rate (7%) in the factory population.

In addition, the tuberculin macrophage test was only positive in one of six patients with tuberculosis and in only two of 45 of the control group.

The modified tuberculin macrophage migration inhibition test in patients with tuberculosis was positive in only two (0·4 and 0·74) of 12 using three-day culture supernatants and in only one (0·79) of four using one-day cultures, and were independent of lymphokine and PPD concentrations. In tuberculin skin-positive subjects there were no positive results (0 of 6) using one-day culture supernatants, and only one (0·78) of five was positive with three-day culture supernatants. This test was again therefore unsatisfactory.

Discussion

Our comparison of two in vitro beryllium hypersensitivity tests have demonstrated the advantages of the lymphocyte transformation test. The beryllium transformation test gave a positive result in all seven patients with chronic beryllium disease, and was independent of steroid therapy. In contrast, the Be macrophage test was not reproducible and in three patients, two on steroids, gave negative results.

In healthy potentially exposed workers, from two factories, the Be transformation test was negative in all 72 tested. The Be macrophage test was positive in four of 78 (3%) and of two retested, one was negative. These positive workers were indistinguishable from those with negative results, as regards details of potential exposure and length of employment, and showed no evidence of beryllium or other disease.

Although the two factories were using beryllium in different manufacturing processes, the results of both Be tests and the PPD macrophage test were very similar. Positive PPD transformation tests were, however, fewer in the ceramic factory (50%) than in the machining factory (65%). In the control healthy subjects, the Be macrophage test was positive in two subjects (4%), neither of which had any known beryllium exposure.

The reliability of the tests was also assessed using tuberculin. The PPD transformation test was positive in 58% of the healthy factory workers. This incidence compares with the 60% positive Mantoux reaction found in the factory 1 population.[6] The PPD macrophage test gave an unacceptably low positive rate in the factory population, tuberculosis patients and Mantoux-positive subjects. Attempts to improve the technique by concentrating lymphokine supernatants were ineffective.

Table 4 *Comparison of the macrophage migration inhibition, MIF, and lymphocyte transformation (LT) tests (Be and PPD) in healthy beryllium workers*

Factory	*Positivity*	*Be*		*PPD*	
		MIF	*LT*	*MIF*	*LT*
1	+	2	0	2	26
	−	33	35	26	14
2	+	2	0	3	17
	−	35	37	36	17
Positive rate:		4/72 (5%)	0/72	5/67 (7%)	43/74 (58%)

Our results contrast with those of Rocklin[12] who found the MIF assay of human lymphocyte supernatants, in most instances, a reliable test for assessing tuberculin hypersensitivity. Even so, the sensitivity of the Rocklin technique does not match that of the direct macrophage migration inhibition test for detecting hypersensitivity in experimental animals.[13] Loss of sensitivity may be associated with dialysis of lymphokine preparations which tend to disrupt MIF molecules.[14]

We conclude that the lymphocyte transformation test is superior to the macrophage migration inhibition assay as a means of detection of hypersensitivity using both beryllium and tuberculin antigen. Also, the transformation tests is technically easier and does not require laboratory animals. We therefore advocate the use of the beryllium lymphocyte transformation test in the diagnosis of beryllium disease and in monitoring the health of potentially exposed workers.

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Requests for reprints to: Dr W Jones Williams, Department of Pathology, Llandough Hospital, Penarth, Glam CF6 1XX, Wales.