Failure to Demonstrate Leukocyte Migration Inhibition in Human Tuberculin Hypersensitivity[1] (34339)

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Antigen-specific inhibition of guinea pig peritoneal macrophages by a nondialyzable soluble factor is now well established as an *in vitro* correlate of experimental delayed hypersensitivity (1, 2). Recently an adaptation of this technique to naturally occurring human hypersensitivity has been reported: antigen-specific inhibition of buffy coat cell migration is stated to correlate excellently with skin sensitivity to whole killed brucella bacilli (3, 4). We have used this buffy coat technique with a different antigen and have been unable to confirm these observations.

Materials and Methods. Forty ml of heparinized venous blood were obtained from subjects known to be either skin test sensitive or insensitive to 0.2 μg of purified protein derivative of tuberculin (PPD). The subjects were either normal volunteers (11 expts.) or patients hospitalized with various rheumatic diseases (13 expts.). None of the latter were taking corticosteroid hormones or other immunosuppressant therapy. Whole blood kept at 37° was allowed to sediment; the buffy coat cells were obtained by centrifugation of the supernatant plasma. The cells were then washed four or more times in Hanks' balanced salt solution (Gibco). Excess erythrocytes when present were lysed by brief exposure to hypotonic saline. The differential leukocyte counts after such treatment averaged 42% lymphocytes (range 8–88%) and viability, assessed by trypan blue exclusion, was usually greater than 95% (range 82–100%). The leukocytes were then suspended at a concentration of $2.5\text{–}5.0 \times 10^7$ cells/ml in TC 199 (Gibco) and aspirated into capillary tubes which were then centrifuged, scored with a file, fractured, and placed into duplicate Mackaness-type chambers (5) containing 10% fetal calf serum (Gibco) in TC 199, penicillin and streptomycin, and either no antigen or 40 μg/ml of commercial PPD (Merck, Sharpe and Dohme) or preservative-free PPD (generously supplied by Dr. M. W. Fisher, Parke Davis and Company, Detroit, Michigan), both demonstrated to be biologically potent by *in vitro* and *in vivo* testing of Freund's adjuvant-immunized guinea pigs. Additional control experiments were performed using subjects' own centrifuge-clarified plasma in place of fetal calf serum. Chambers were incubated at 37° for 18–24 hr, at which time cell fans were photographed, the images were projected, and the areas of the images were measured by planimetry. Migration indices were calculated according to the formula:

$$\text{migration index} = \frac{\text{area of migration in presence of antigen}}{\text{area of migration in absence of antigen}}.$$

Results. Fourteen tests were performed on persons with negative tuberculin reactions and 10 on persons with positive reactions. The results are shown in Table I and demonstrate poor concordance between skin and *in vitro* tests. The mean migration index for tuberculin-negative subjects was 0.84 and was not significantly different from that for tuberculin-positive subjects, 0.77. Overlap was considerable. In this and a larger series of parallel experiments it was demonstrated that results were not influenced by cell viability, percentage erythrocyte contamination (with-

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TABLE I. Buffy Coat Migration Index in PPD Negative and Positive Subjects.

Subject	Antigen[a]	Migration index
Negative skin tests		
1.	C	0.85
2.	C	0.84
3.	C	0.73
4.	C	0.75
5.	C	1.09
6.	C	0.78
7. a	C	0.99
b	C	0.91
c	C	0.78
d	PF	0.94
e	PF	0.61
f	PF	0.88
8.	PF	0.73
9.	PF	0.83
Av, all skin test negative		0.84
Positive skin tests		
10.	C	0.57
11. a	C	0.39
b	PF	0.74
12. a	PF	0.37
b	PF	1.06
13.	PF	0.99
14.	PF	0.77
15.	PF	1.01
16.	PF	0.91
17.	PF	0.87
Av, all skin test positive		0.77

[a] C = commercial PPD; PF = preservative-free PPD.

in the experimental range), percentage lymphocytes present, size of tuberculin reaction, type of PPD (at a concentration of 40 μg/ml) or diagnosis of donor. Repeated and duplicate experiments on the same subject demonstrated approximately 10% variability between individual capillary cell fan areas within an experiment, but considerably more variability between migration indices between successive experiments. Experiments using the subjects' own fresh unheated plasma did not predictably alter the results but suggested that plasma could magnify the inhibition seen in some subjects. Cross experiments using plasma from one subject and cells from another were unsuccessful because inhibition occurred in both control and antigen-containing chambers. The isolated low migration indices seen in subject 7 both occurred when blood was obtained on the third day following a (negative) tuberculin skin test; this effect cannot explain other low results, however, since leukocytes were usually obtained prior to skin testing.

Discussion. Although some reproducible positive results were obtained, migration-inhibition of buffy coat cells does not in our hands reliably correlate with skin-test sensitivity. Four protocol differences may explain why our observations differ from those of the brucella experiments of Søborg. First, we did not use "subtoxic" (6) antigen concentrations, as measured by trypan blue exclusion. However the *in vitro* antigen concentration was 20 or more times the eliciting *in vivo* antigen concentration in our system in contrast to 2.5 times that of the skin test in the brucella experiments. Other of our experiments using concentrations up to 100 μg/ml of preservative-free PPD did not increase border-line inhibition nor decrease cell viability. Stimulation of migration, expected if the antigen concentrations were too low (6, 7), was not observed.

Second, we did not use a particulate antigen. However our antigen was effective in an animal model and nonparticulate antigens have been successfully used by the same authors who used brucella bacilli (8–11). Third, not all our skin-test positive cell donors were strong reactors to PPD; nonetheless 2 or 4 persons with necrotizing skin reactions, subjects 13 and 15, had clearly negative *in vitro* tests. Fourth, we found that erythrocyte contamination sufficient to color the cell button caused marked cell spillage, and so we subjected the erythrocytes to hypotonic lysis. Such treatment has not, in our hands, influenced the guinea pig macrophage prototype model, and both positive and negative results were obtained with human buffy coat cells whether or not lysis was performed.

In other respects our protocol was patterned after Søborg's (3). It is therefore unlikely but not ruled out that systematic artifacts, such as persistence of serum antibody in the media bathing the cells (12), could account for the differences between our results.

An active and nondialyzable migration inhibitory substance can be obtained from human buffy coat lymphocytes incubated with PPD (13, 14) whether or not polymorphonuclear leukocytes are present (15). Hence we suspect that our failure to demonstrate inhibition of buffy coat leukocytes is due not to a failure of migration inhibitory factor production but to inadequate response of healthy human leukocytes to PPD-induced inhibitory factor. We have not excluded the possibility that nonspecific injury to the leukocytes, such as might be caused by brucella toxins (16), might render the leukocytes more sensitive to inhibitory factor; if so, this requirement of toxicity would be unique to the cell migration-inhibition models described to date. Our results indicate that, unlike guinea pig peritoneal exudate assays, human peripheral blood buffy coat cell migration-inhibition does not consistently reflect skin test sensitivity to PPD and therefore cannot be unqualifiedly said to be a correlate of human delayed hypersensitivity.

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