Immunology 1980 **41** 227

Effects of human transfer factor on the migration of guinea-pig macrophages: is there an antigen-specific activity?

I. L. SARGENT* & M. R. SALAMAN *Department of Immunology, St Mary's Hospital Medical School, London*

Accepted for publication 10 April 1980

Summary. Dialysable transfer factor (TF) prepared from the buffy-coat cells of tuberculin-positive human donors exerted antigen (PPD)-dependent inhibition of migration of guinea-pig peritoneal exudate cells (PEC) provided that migration of the cells was not strongly affected by PPD alone. TF from tuberculin-negative donors did not do this. The effect could be better demonstrated with tuberculin-sensitive than with normal PEC. Differences in the actions of 'tuberculin-positive' and 'negative' TF may also be seen in the absence of antigen. In a similar but more restricted series of experiments with diphtheria toxoid (DT) as antigen, DT-dependent inhibition was observed only with 'DT-positive' TF. The findings concerning antigen-dependent inhibition in both the tuberculin and toxoid systems are compatible with the concept of an antigen-specific TF, but it is argued that they should not be taken as strong evidence of such specificity. In the tuberculin system the results suggest an alternative explanation, namely that 'tuberculin-positive' TF contains a higher level of a non-specific activity. Whether specific or not, the antigen-dependent activity probably involves a stimulatory action on antigen-induced lymphocyte activation leading to enhanced production of macrophage migration inhibition factor, and it could be related to the 'transfer' phenomenon *in vivo*.

* Present address: Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, Oxford.

Correspondence: Dr M. R. Salaman, Department of Immunology, St Mary's Hospital Medical School, London W2 1PG.

0019-2805/80/0900-0227$02.00

INTRODUCTION

The appearance of delayed hypersensitivity towards environmental and other antigens in man following the injection of dialysable extracts of human leucocytes is attributed to the presence in such extracts of 'transfer factor' (TF) (Lawrence, 1974). However, it is not clear whether this phenomenon represents a true transfer of sensitivity from a sensitive donor to a negative recipient or whether it is brought about by non-specific amplification of low-level sensitivity pre-existing undetected in the recipient (Bloom, 1973; Salaman & Valdimarsson, 1976).

Because of the restrictions of human experimentation, this problem is probably best approached by use of *in vitro* systems. Starting from the hypothesis that TF is an antibody-like molecule, Salaman (1974) studied the action of human TF on the migration of peritoneal exudate cells (PEC) from normal guinea-pigs. TF from tuberculin-positive donors was found to inhibit migration in the presence of antigen (PPD), and the effect was not observed with TF from negative donors. This is consistent with an antigen-specific TF, and the system held out the promise of resolving the issue of specificity.

There were, however, difficulties with this system. Consistent antigen-dependent inhibition by 'tuberculin-positive' TF could be obtained using PPD at 8 μg/ml, but the extent of inhibition was small. For strong antigen-dependent inhibition a higher level of PPD (20 μg/ml) was necessary. At this concentration, however, PPD frequently enhanced or inhibited mig-

ration in the absence of TF, and the strong antigen-dependent inhibition was restricted to about half the experiments in which PPD had little effect alone.

In the present study we have attempted to get round these problems by various stratagems. As described later, the method of preparation of human TF was modified in a way which had been reported to provide greater consistency in a related system (Ascher, Schneider, Valentine & Lawrence, 1974). Such preparations from tuberculin-positive and negative donors were tested both on normal PEC and, with a view to controlling the effect of PPD alone on migration, on PEC from tuberculin-sensitive guinea-pigs. Experiments were also carried out with TF from donors of known sensitivity to diphtheria toxoid (DT). It was hoped that DT, unlike PPD, would have no effect on migration in the absence of TF. Moreover, an investigation of antigen-specificity in TF demanded the use of more than one antigen.

MATERIALS AND METHODS

Sensitivity of transfer factor donors

Human (adult) donors of blood for preparation of TF had defined cell-mediated reactivity to PPD and, where appropriate, to DT. The sensitivity data will be presented briefly here; full details are recorded elsewhere (Salaman, 1978). Skin-test results are given as mm of induration at 48 h following injection of 0·2 μg of PPD or a Schick-test dose of DT. Lymphocyte transformation to PPD and DT was tested at 1 μg/ml and 2 Lf units/ml, respectively. Transformation index is the ratio of incorporation of tritiated thymidine in the presence of antigen to that occurring in its absence, at the end of a 6 day culture period.

A group of strong tuberculin responders reacted as follows: BW gave 30 mm (induration) and 50 (transformation index); MC 20 mm and 81; VW 15 mm and 15; LB 20 mm and 74. A tuberculin-negative group showed zero induration and no more than a trace of erythema at 2 μg PPD. Their transformation indices were PC 2·8, AB 1·7 and MR 4·9.

JD gave a massive delayed skin reaction (65 mm) to DT and indices of 31 (DT) and 17 (PPD). MC and PC gave indices (DT) of 1·3 and 9, respectively.

Preparation of transfer factor

TF was prepared from the buffy-coat cells of 30 ml samples of blood as described previously (Salaman, 1978). The cell lysates produced by freezing and thawing were dialysed overnight at 4° against RPMI containing antibiotic (see details of medium below). Dialysates were sterilized by filtration and used on the same day in one or two experiments, that is with one or two batches of PEC. Thus donors were bled a number of times equivalent to at least half the experiments performed with their TF. Variation in TF activity between experiments for a particular donor was similar whether the same or different preparations were used.

Collection of peritoneal exudate cells

PEC were collected 3 days after injection of oil into normal or tuberculin-sensitive Hartley guinea-pigs (Salaman, 1974).

Sensitization was carried out as follows. Freund's adjuvant containing *M. tuberculosis* strain H37 Ra (Difco, Detroit, U.S.A.) was emulsified with an equal volume of saline. A total volume of 0·5 ml was injected s.c. in the left and right axillary and inguinal regions. PEC were collected at various times after sensitization.

Migration of peritoneal exudate cells

Capillary migration of PEC was carried out in Sterilin plastic migration chambers as described previously (Salaman, 1974). Migration areas were read at 18 h (experiments with PPD as antigen) or 6 h (experiments with DT). Ratios between areas obtained at 6 h showed little difference from corresponding ratios at 18 h, but the areas themselves were approximately three times as great at the later time.

Culture media were based on RPMI 1640 (Flow Laboratories) with penicillin and streptomycin at 50 units/ml. Where TF dialysate was present it replaced an equivalent volume of RPMI. Antigens PPD and DT were added as concentrates in RPMI. All media contained 13% normal guinea-pig serum that had been heated at 56° for 30 min. Media were gassed with 5% CO_2 prior to addition to the chambers.

TF was used at a concentration of 5×10^6 white blood cell (WBC) equivalents/ml or as indicated. Mononuclear leucocytes represented 25–45% of WBC.

PPD was a freeze-dried preparation of human tuberculin (Ministry of Agriculture, Weybridge). DT (Wellcome) was dialysed exhaustively against phosphate-buffered saline. Antigens were used at 5, 10 or 20 μg/ml (PPD) and 2 or 10 Lf units/ml (DT).

Analysis of migration areas

Each group was represented by six capillaries and the

mean migration area was obtained as the square of the root mean of the individual areas.

In each experiment the mean migration areas were used to calculate certain ratios. The antigen–control ratio (ACR) is the ratio of the mean migration area obtained in the presence of antigen to that obtained in medium alone, multiplied by 100. The TF–control ratio (TFCR) is the ratio of the mean area obtained in the presence of TF to that obtained in medium alone, multiplied by 100. The antigen–test ratio (ATR) is the ratio of the mean area obtained in the presence of TF and antigen to that obtained in the presence of TF, multiplied by 100.

ATR was used in examining the question of antigen-dependent inhibition by TF. Values of ATR were plotted against corresponding values of ACR, and a line of unit gradient was drawn through the origin (Figs 3 and 4). If TF was doing nothing in the system that was antigen-dependent, the points would be expected to fall symmetrically about the line. Antigen-dependent inhibition would be revealed by a tendency for the points to fall below the line. The distribution of points may be described both by the ratio of the number of points above to that below the line (PDR) and by their mean vertical distance from the line (MVD). In calculating PDR, points on the line were ignored. The vertical distances were obtained directly from the values in the figures, points above or below the line giving rise to positive or negative values respectively (the distances are equivalent to ATR-ACR). MVD is given $\pm$ standard deviation.

A value of MVD significantly less than zero indicates antigen-dependent inhibition, and this was tested by the Wilcoxon ranking procedure. The same test was used to answer the question whether there was a difference in the behaviour of TF from sensitive and negative donors, the parameter tested being the difference between ATR for 'positive' and 'negative' TF within each experiment. Where there were more than one preparation of the same type in an experiment mean values of ATR were used.

When means of ratios were required these were obtained as the square of the root mean of the individual ratios.

RESULTS

Antigen-dependent and independent effects of TF from donors of known tuberculin sensitivity on the migration of PEC from normal guinea-pigs

Experiments to test the effect of TF from tuberculin-positive and negative donors on the migration of normal PEC in the presence and absence of PPD have been carried out by Salaman (1974), and the problems that were encountered are described in the Introduction. In that study, TF was prepared by dialysis of cell lysates against water; the dialysates were lyophilized and taken up in culture medium for testing. Subsequently, Ascher *et al.* (1974) reported that an *in vitro* stimulatory effect of TF on antigen-induced lymphocyte transformation was more consistently observed if TF was prepared by dialysing directly into culture medium. It was decided therefore to repeat the migration experiments with their method of preparation.

Medium-dialysed TF was tested from several tuberculin-positive and negative donors at $1{\cdot}5–12 \times 10^6$ WBC equivalents/ml, and PPD was used at 10 and 20 μg/ml. It soon became clear that the results were falling into a pattern similar to that of the earlier work and that no advantage had been gained. Thus, modest antigen-dependent inhibition was generally observed with 'tuberculin-positive' TF when PPD alone had little effect on migration. The two antigen levels gave similar results except that the higher level was more likely to affect migration on its own.

New information was obtained from experiments in which the effect on migration of TF alone (TFCR) was tested at two or more concentrations (Fig. 1). For 'tuberculin-positive' TF increasing the concentration invariably resulted in greater inhibition or reduced enhancement. However, 'negative' TF did not behave in this way, higher levels usually producing greater enhancement or reduced inhibition.

Antigen-dependent and independent effects of TF from donors of known tuberculin sensitivity on the migration of PEC from sensitized guinea-pigs

The experiments carried out with PEC from tuberculin-sensitive guinea-pigs are detailed in Table 1. PEC were collected from 7 to 50 days after sensitization. Medium-dialysed TF was used at 5×10^6 WBC equivalents/ml and PPD at 5 and 20 μg/ml.

Figure 2 shows effects of the high level of antigen alone (ACR) plotted against the corresponding values of TFCR. It is seen that ACR depends on the time interval between sensitization and collection of PEC, ranging from no inhibition of migration on day 7 to over 60% inhibition on day 50. In contrast there was no significant correlation between TFCR and either time interval or ACR. Thus, TF from tuberculin-positive or negative donors gave mean values of

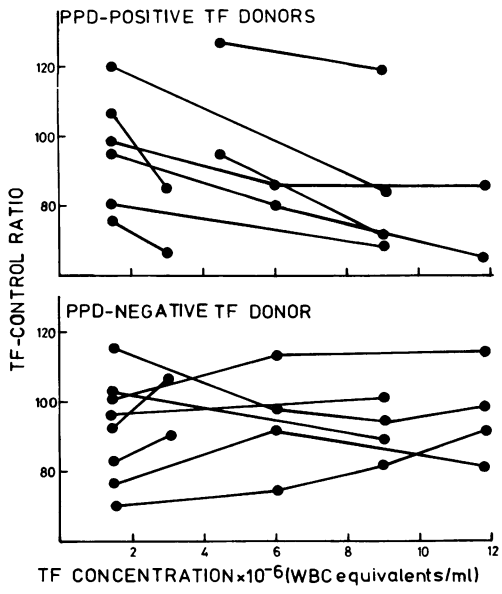

Figure 1. Concentration dependence of the effect of TF from tuberculin-positive or negative donors on the migration of PEC from normal guinea-pigs. The values of TF-control ratio (TFCR) obtained at different concentrations of a preparation in the same experiment are joined by a line. TF came from PC (negative) or from the following positive donors. Reading from top to bottom at a TF level of $1{\cdot}5 \times 10^6$ WBC equivalents/ml the donors were VW, BW, MC, MC, VW and BW. MC was the donor for both experiments starting at $4{\cdot}5 \times 10^6$ equivalents/ml.

TFCR at 7, 16, 25 and 50 days all in the range 89–93, and it clearly has a tendency to effect slight inhibition at this concentration. Mean values of TFCR at this same concentration in experiments with normal PEC were 94 ('positive') and 96 ('negative').

Antigen-dependent effects of TF on sensitised PEC are shown in Fig. 3. For 'negative' TF it is clear that no such effects occurred, values of ATR being closely similar to corresponding values of ACR throughout the entire range. On the other hand, 'positive' TF exerted antigen-dependent inhibition when the effect of antigen alone was small (ACR above 85). In this range, values of PDR and MVD were respectively 0·22 and $-9{\cdot}1 \pm 12{\cdot}7$ ('positive' TF) and 0·75 and $-0{\cdot}7 \pm 7{\cdot}8$ ('negative' TF). This represents significant antigen-dependent inhibition by 'positive' TF ($P=0{\cdot}01$) and a significant difference in the activity of 'positive' and 'negative' preparations ($P=0{\cdot}05$).

The above values of PDR and MVD are 'overall' values in that they incorporate all the points in the appropriate range irrespective of antigen concentration. If the effects at the two antigen levels are analysed separately, significant antigen-dependent inhibition by 'positive' TF is established only at 20 μg/ml of PPD [PDR = 0; MVD = $-13{\cdot}7 \pm 14{\cdot}6$ ($P=0{\cdot}01$)]. It seems that the best conditions for antigen-dependent inhibition occur when antigen alone has a slight inhibitory action as opposed to no effect at all, and that it is this requirement rather than antigen concentration as such which is important.

Table 1. Effect of TF from donors of known tuberculin sensitivity on the migration of PEC from sensitized guinea-pigs in the presence and absence of PPD

Expt No.	PEC (days after sensitization)	TF donor	Mean migration areas		
			PPD absent	PPD present	
				5 μg/ml	20 μg/ml
1	7	—	1818 (1723–1914)	1938 (1843–2035)	1896 (1839–1954)
		BW	1530 (1434–1629)	1660 (1548–1776)	1358 (1235–1485)
		MC	1473 (1289–1670)	1571 (1344–1815)	1494 (1360–1633)
		MR	1618 (1536–1702)	1759 (1627–1897)	1653 (1520–1793)
2	7	—	1283 (1160–1411)	1382 (1215–1561)	1293 (1177–1413)
		BW	1257 (1164–1353)	1274 (1192–1359)	1242 (1152–1335)
		MC	1193 (1089–1303)	1294 (1229–1361)	1170 (1093–1251)
		MR	1252 (1173–1334)	1425 (1285–1573)	
3	16	—	1702 (1609–1797)		1187 (1083–1296)
		BW	1566 (1455–1681)		1016 (953–1079)
		MC	1276 (1153–1406)		1031 (943–1122)
		MR	1326 (1202–1456)		1138 (1052–1227)

Table 1 (continued)

4	16	—	1288 (1144–1441)		1106 (1081–1130)
		BW	1497 (1335–1669)		987 (841–1144)
		MC	1172 (1073–1275)		890 (773–1016)
		MR	1344 (1127–1580)		1281 (1066–1516)
5	25	—	1555 (1410–1707)	1132 (996–1276)	812 (701–931)
		MC	1672 (1437–1924)	1001 (912–1095)	781 (688–880)
		PC	1508 (1303–1728)	963 (891–1038)	797 (716–882)
6	25	—	851 (732–979)	809 (683–945)	820 (669–987)
		BW	1090 (940–1251)	833 (716–959)	581 (446–734)
		PC	880 (804–960)	831 (673–1006)	713 (573–868)
7	25	—	1561 (1443–1685)		694 (600–796)
		BW	1364 (1278–1453)		701 (606–823)
		MC	1593 (1538–1649)		742 (661–828)
		MR	1410 (1352–1470)		760 (667–859)
8	25	—	1234 (1054–1428)		433 (245–674)
		BW	959 (847–1077)		490 (381–614)
		MC	692 (467–960)		429 (388–473)
		MR	1035 (874–1280)		351 (286–422)
9	25	—	980 (756–1234)	521 (412–643)	
		BW	830 (602–1094)	595 (514–682)	
		PC	995 (702–1339)	469 (394–549)	
10	25	—	870 (651–1122)	926 (778–1085)	
		BW	854 (711–1009)	935 (809–1070)	
		PC	1071 (855–1312)	1094 (982–1211)	
11	25	—	892 (654–1166)	670 (514–847)	
		BW	1064 (910–1229)	816 (712–926)	
		PC	913 (843–985)	550 (448–663)	
12	50	—	1579 (1510–1649)		552 (483–626)
		BW	1599 (1496–1704)		518 (447–593)
		PC	1503 (1360–1654)		534 (470–603)
13	50	—	1415 (1343–1489)		490 (409–578)
		BW	1174 (1054–1300)		439 (357–529)
		PC	1345 (1259–1434)		450 (353–560)
14	50	—	883 (726–1054)		191 (124–274)
		BW	807 (696–927)		173 (162–184)
		PC	841 (614–1104)		165 (109–233)
15	50	—	861 (752–977)		231 (169–303)
		BW	755 (637–883)		193 (135–263)
		PC	768 (628–922)		170 (114–236)

PEC were collected from guinea-pigs that had been injected with Freund's adjuvant containing killed *M. tuberculosis* at the times shown, and the effect on their migration of TF and PPD, alone and together, was determined. The mean migration areas at 18 h with the range of one standard deviation are given in arbitrary planimeter units. TF was used at 5×10^6 WBC equivalents/ml from BW and MC (tuberculin-positive) and PC and MR (negative) (see Methods).

When ACR was less than 60, representing strong inhibition by antigen, 'positive' TF was sometimes able to reduce the extent of such inhibition (Fig. 3). This is reflected by overall PDR and MVD values of 1·5 and $+6{\cdot}4 \pm 10{\cdot}7$, respectively. The values for 'negative' TF are 0·6 and $-0{\cdot}4 \pm 4{\cdot}8$.

Antigen-dependent effects of TF from donors of known diphtheria toxoid sensitivity on the migration of PEC from normal guinea-pigs

These experiments with normal PEC are detailed in Table 2. Medium-dialysed TF was used at 5×10^6

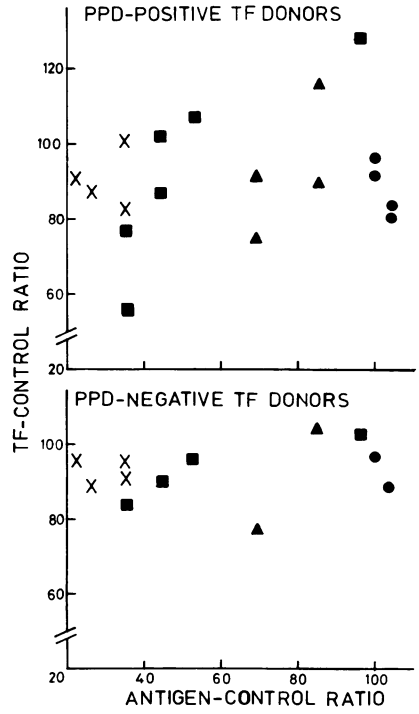

Figure 2. Relationship between the effect of TF from tuberculin-positive or negative donors and that of PPD on the migration of sensitized PEC. TFCR at a TF concentration of 5×10^6 WBC equivalents/ml is plotted against corresponding values of antigen-control ratios (ACR) at 20 μg/ml of PPD. TF came from BW and MC (positive) or PC and MR (negative) (see Table 1). PEC were collected at various times after sensitization with killed *M. tuberculosis*:- (●) 7 days, (▲) 16 days, (■) 25 days and (×) 50 days.

WBC equivalents/ml and the antigen was DT at 2 and 10 Lf units/ml. TF came from JD (highly sensitive to DT) or from PC (moderate sensitivity) and MC (negative) (see Methods).

ATR has been plotted against corresponding values of ACR in Fig. 4. There was little effect of DT alone at the lower concentration, but at the higher level strong inhibition was observed in two of six experiments. Nevertheless, for 'DT-positive' TF (donor JD) all points in Fig. 4 fell below the line of unit gradient representing highly significant antigen-dependent inhibition [PDR=0; MVD=$-16{\cdot}3 \pm 11{\cdot}5$ ($P<0{\cdot}01$)]. Significant inhibition is also established treating points obtained at the two different antigen levels independently (in each case $P<0{\cdot}025$). By contrast, the other preparations gave points scattered about the line. For donor PC the overall parameters were respectively 0·5 and $-3{\cdot}2 \pm 21{\cdot}0$; for MC they were 1·0 and $-4{\cdot}2 \pm 37{\cdot}7$. The points obtained with MC were particularly widely scattered.

DISCUSSION

Inhibition of migration that was dependent on the presence of antigen was demonstrated only with TF from sensitive donors whether in the tuberculin or diphtheria toxoid systems, and this is compatible with the concept of an antigen-specific TF. However, these observations should not be taken as providing strong evidence of specificity.

As regards the tuberculin system, there is considerable support for an alternative interpretation, namely

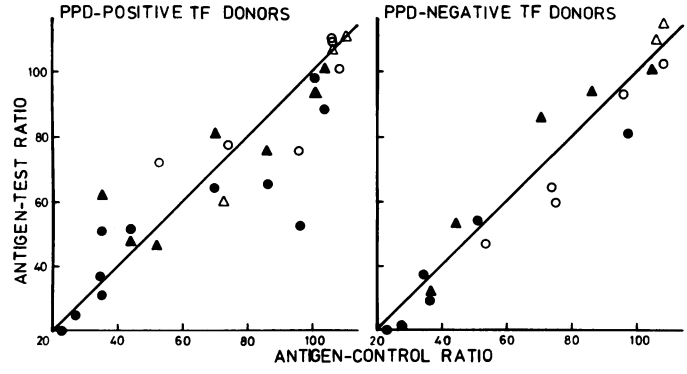

Figure 3. Antigen-dependent effects of TF from donors of known tuberculin sensitivity on the migration of sensitized PEC. ACR at 5 μg/ml of PPD (open symbols) and 20 μg/ml (closed symbols) is plotted against corresponding values of antigen-test ratio (ATR). TF at 5×10^6 WBC equivalents/ml came from positive donors BW (circles) and MC (triangles) or negative donors PC (circles) and MR (triangles). PEC were collected from 7 to 50 days after sensitization (see Table 1).

Table 2. Effect of TF from donors of known sensitivity to diphtheria toxoid (DT) on the migration of normal PEC in the presence and absence of DT

Expt No.	TF donor	Mean migration areas		
		DT absent	DT present	
			2 Lf u/ml	10 Lf u/ml
1	—	519 (355–713)		374 (320–433)
	JD	639 (470–835)		425 (331–530)
	MC	624 (545–709)		340 (308–375)
2	—	569 (425–735)		336 (257–426)
	JD	472 (331–637)		256 (189–334)
	MC	597 (548–649)		431 (369–499)
3	—	440 (365–523)	500 (351–676)	513 (432–601)
	JD	537 (438–647)	511 (438–590)	425 (329–534)
	MC	663 (579–752)	525 (372–705)	409 (294–543)
	PC	521 (426–625)	581 (492–678)	447 (363–538)
4	—	341 (273–416)	326 (246–416)	304 (211–415)
	JD	371 (240–531)	338 (275–407)	271 (226–320)
	MC	199 (114–308)	258 (167–369)	248 (165–348)
	PC	300 (212–403)	287 (194–397)	328 (253–413)
5	—	790 (694–892)	773 (670–883)	877 (728–1040)
	JD	963 (842–1090)	830 (726–943)	737 (671–805)
6	—	685 (575–804)	766 (708–827)	719 (616–830)
	JD	673 (590–760)	661 (567–762)	608 (577–640)

PEC were collected from normal guinea-pigs, and the effect on their migration of TF and DT—alone and together—was determined. Mean migration areas at 6 hours are given as in Table 1. TF was used at 5×10^6 WBC equivalents/ml from JD (strongly sensitive to DT), PC (moderate sensitivity) and MC (negative) (see Methods).

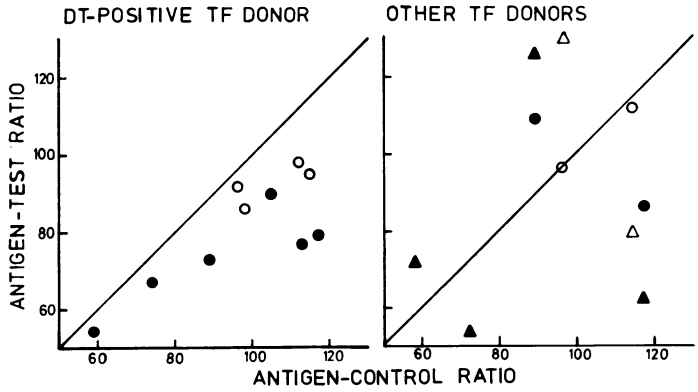

Figure 4. Antigen-dependent effects of TF from donors of known sensitivity to DT on the migration of normal PEC. ACR at 2 Lf units/ml of DT (open symbols) and 10 Lf units/ml (closed symbols) is plotted against corresponding values of ATR. TF at 5×10^6 WBC equivalents/ml came from the strongly DT-positive donor JD (circles in left-hand graph) or from PC (circles in right-hand graph) and MC (triangles) showing respectively moderate and zero sensitivity.

that TF preparations from tuberculin-positive donors contain a higher level of a non-specific activity than do those from negative donors. In the first place, the clearest demonstration of PPD-dependent inhibition of migration by 'tuberculin-positive' TF came in experiments with PEC from tuberculin-sensitive guinea-pigs. This can hardly involve specific transfer of sensitivity. The effect was seen when PPD alone brought about some inhibition of migration but not more than 15%. So it seems that 'positive' TF is able to amplify a small inhibitory response to PPD. Antigen-dependent inhibition of migration of normal PEC by 'positive' TF is probably brought about by the same mechanism since such cells frequently exhibit a naturally-acquired low-level sensitivity to PPD (present results and Salaman, 1974). The effect is again seen only when that of PPD alone is small. It seems likely then that 'positive' TF contains a non-specific factor which promotes either the production of MIF from activated lymphocytes or the responsiveness of macrophages to MIF.

A second reason for favouring non-specificity concerns the demonstration of differences between TF from tuberculin-positive and negative donors in their effects on migration of normal PEC in the *absence* of PPD (Fig. 1 and Salaman, 1974). Having observed differences in non-specific activity affecting migration, such activity must be a strong candidate for bringing about the observed difference in antigen-dependent activity. This line of argument would be undermined if specific antigen is present in TF preparations from sensitive donors, but there is no evidence that this is normally the case (Salaman, 1978). Thus, 'positive' TF produced no marked inhibition of migration of sensitive PEC and behaved similarly to 'negative' TF (Fig. 2). The previously observed correlation between the action of 'positive' TF and that of PPD on normal PEC should not be ascribed to the presence of antigen in TF (Salaman, 1974). It seems more likely to reflect an instability in that system such that any mild 'stimulant' might induce slight inhibition or enhancement. The direction would be characteristic of the particular batch of PEC and hence the effects of TF and antigen similar within an experiment.

TF from tuberculin-positive donors, as compared to 'negative' TF, has also been shown to be more active in enhancing in a non-specific manner antigen-induced transformation of lymphocytes (Salaman, 1978). Possible reasons for these differences in non-specific activity are being investigated and will be discussed in detail in a later paper. In Britain, where BCG is given at school, there may be significant reasons for the absence of hypersensitivity in adults. On the other hand, a feature of strong tuberculin sensitivity may be the presence of a granuloma, and the release of mediators from this source could conceivably affect leucocytes throughout the body so as to increase TF activity.

Similar findings to those presented here have been made by Dabrowska, Hamblin & Dumonde (1976) in experiments with normal guinea-pig PEC and mixtures of normal PEC and human lymphocytes. The latter system is comparable to our system of sensitive PEC in that the effect of PPD alone, dependent as it is on the tuberculin sensitivity of the lymphocyte donor, can be controlled. Antigen-dependent inhibition by 'tuberculin-positive' TF occurred in both their systems when there was little inhibitory effect of PPD. 'Negative' TF was tested in the un-mixed cell system and showed less activity. In both systems, when PPD alone strongly inhibited migration, TF abrogated this effect. The same strange observation was made in our studies with normal PEC (Salaman, 1974) and now, though it was less pronounced, with sensitive PEC. This is another situation where 'positive' TF was more potent than 'negative'. A similar abrogation effect has been observed with human buffy-coat cells (Borkowsky & Lawrence, 1979).

Turning to the toxoid system, preparations from a donor with strong sensitivity to DT produced small but reproducible DT-dependent inhibition of migration of normal PEC, and this was not so with those from a moderate or non-responder to this antigen. The finding cannot be explained on the basis of tuberculin sensitivity since the DT-negative donor had the highest level of that sensitivity. Interestingly, this donor produced marked DT-dependent effects, both inhibitory and enhancing. However, no firm conclusions can be drawn on specificity in the DT system until TF from several strongly DT-positive and negative donors, whose tuberculin sensitivities are known, have been tested both against DT and PPD.

In view of these results with DT, and recent evidence from migration of human buffy-coat cells (Borkowsky & Lawrence, 1979; Wilson, Fudenberg & Horsmanheimo, 1979) and from *in vivo* 'transfer' (Kirkpatrick, 1978; Burger, Vandenbark Dunnick, Kraybill, Daves & Vetto, 1979), it is not possible to discard the concept of specificity. However, any specific factor would be co-existing alongside undoubted non-specific *in vivo* amplification activity (Vandenbark, Burger & Vetto, 1977; Wilson, Welch & Fudenberg, 1977; Salaman,

Sargent & Sljivic, 1979; Gottlieb, Maziarz, Tamaki & Sutcliffe, 1980). The augmentation of antigen-induced migration inhibition demonstrated in the tuberculin system is likely to be a manifestation of this last activity. But it could just possibly be a specific augmentation brought about through the presence in 'positive' TF of a factor which binds antigen forming a complex with enhanced efficiency in lymphocyte activation. The major difficulty is to explain how such an antibody-like factor could be dialysable (Salaman, 1974).

ACKNOWLEDGMENTS

We are most grateful to Miss Jane Robinson and Mr David Davies for technical assistance. We thank Dr Anne Hamblin and Professor John Humphrey for helpful discussions and Professor Leslie Brent and Drs Vivian Rumjanek and Helgi Valdimarsson for their suggestions on the manuscript. The work was supported by grants from the Medical Research Council and the Wellcome Trust. I.L.S. held a scholarship from the Wellcome Trust.

REFERENCES

ASCHER M.S., SCHNEIDER W.J., VALENTINE F.T. & LAWRENCE H.S. (1974) *In vitro* properties of leucocyte dialysates containing transfer factor. *Proc. natn. Acad. Sci. (U.S.A.)*, **71**, 1178.

BLOOM B.R. (1973) Does transfer factor act specifically or as an immunologic adjuvant? *N. Engl. J. Med.* **288**, 908.

BORKOWSKY W. & LAWRENCE H.S. (1979) Effect of human leucocyte dialysates containing transfer factor in the direct leucocyte migration inhibition (LMI) assay. *J. Immunol.* **123**, 1741.

BURGER D.R., VANDENBARK A.A., DUNNICK W., KRAYBILL W., DAVES G.D. & VETTO R.M. (1979) Human transfer factor: structural properties suggested by HPRP chromatography and enzymatic sensitivities. *J. Immunol.* **122**, 1091.

DABROWSKA B., HAMBLIN A. & DUMONDE D.C. (1976) Potentiating effect of transfer factor upon macrophage migration inhibition *in vitro*. In: *Transfer Factor: Basic Properties and Clinical Applications* (Ed. by M. S. Ascher, A. A. Gottlieb and C. H. Kirkpatrick), p. 147. Academic Press, New York.

GOTTLIEB A.A., MAZIARZ G.A., TAMAKI N. & SUTCLIFFE S.B. (1980) The effects of dialyzable products from human leucocyte extracts on cutaneous delayed-hypersensitivity response. *J. Immunol.* **124**, 885.

KIRKPATRICK C.H. (1978) Transfer of delayed cutaneous hypersensitivity with transfer factor. *Cell. Immunol.* **41**, 62.

LAWRENCE H.S. (1974) Transfer factor in cellular immunity. *Harvey Lectures*, **68**, 239.

SALAMAN M.R. (1974) Studies on the transfer factor of delayed hypersensitivity. Effect of dialysable leucocyte extracts from people of known tuberculin sensitivity on the migration of normal guinea-pig macrophages in the presence of antigen. *Immunology*, **26**, 1069.

SALAMAN M.R. (1978) An investigation into the antigen-specificity of transfer factor in its stimulatory action on lymphocyte transformation. *Immunology*, **35**, 247.

SALAMAN M.R. & VALDIMARSSON H. (1976) Specificity of transfer factor. *Nature (Lond.)*, **259**, 250.

SALAMAN M.R., SARGENT I.L. & SLJIVIC V.S. (1979) Transfer factor as an adjuvant for antibody production in the mouse. In: *Immune Regulators in Transfer Factor* (Ed. by A. Khan, C. H. Kirkpatrick and N. O. Hill), p. 661. Academic Press, New York.

VANDENBARK A.A., BURGER D.R. & VETTO R.M. (1977) Human transfer factor activity in the guinea-pig: absence of antigen specificity. *Clin. Immunol. Immunopathol.* **8**, 7.

WILSON G.B., FUDENBERG H.H. & HORSMANHEIMO M. (1979) Effects of dialyzable leucocyte extracts with transfer factor activity on leucocyte migration *in vitro*. I. Antigen-dependent inhibition and antigen-independent inhibition and enhancement of migration. *J. lab. clin. Med.* **93**, 800.

WILSON G.B., WELCH T.M. & FUDENBERG H.H. (1977) Tx: a component in human dialyzable transfer factor that induces cutaneous delayed hypersensitivity in guinea-pigs. *Clin. Immunol. Immunopathol.* **7**, 187.