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An investigation into the antigen-specificity of transfer factor in its stimulatory action on lymphocyte transformation

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Summary. Dialysable transfer factor (TF) was prepared from the buffy-coat cells of donors with known cell-mediated reactivity to tuberculin (PPD), streptococcal protein (SKSD) and diphtheria toxoid (DT). The effect of such preparations on the transformation by these antigens of lymphocytes from tuberculin-negative donors was investigated. Transformation was determined as incorporation of tritiated thymidine. The concentrations of SKSD and DT were adjusted for different lymphocyte donors so as to give, in the absence of TF, a low index of transformation (less than 10-fold) comparable to that obtained with PPD.

TF from tuberculin-positive donors stimulated antigen-induced transformation by on average approximately 2-fold whereas TF from tuberculin-negative donors generally had little effect. This was so not only for PPD as antigen but also for SKSD and DT, and sensitivity of TF donor to SKSD or DT was not a determining factor. TF also frequently increased background transformation in the absence of antigen. Although a small effect, this ability tended to reflect the activity of TF in the presence of antigen.

It is concluded that neither the whole nor any significant part of this enhancement of transformation can be ascribed to an antigen-specific factor. Tuberculin-positive donors apparently yield a higher level of non-specific factor and possible reasons for this are discussed. The factor active in transformation may be responsible for the TF phenomenon *in vivo*.

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INTRODUCTION

Twenty five years after the discovery of the transfer factor phenomenon it is still not clear whether transfer factor (TF) carries antigen specificity: are the delayed-type hypersensitivities that appear in the recipient following injection of TF specific for donor sensitivities? Much evidence has been collected in support of specificity, but owing to the restrictions of human experimentation an unequivocal answer has not been possible (Bloom, 1973; Salaman & Valdimarsson, 1976).

An *in vitro* activity in TF preparations which seemed likely to be relevant to this phenomenon was described by Ascher, Schneider, Valentine and Lawrence in 1974. They found that a transformation response of considerable magnitude took place when lymphocytes from a skin-test negative donor were cultured in the presence of antigen and TF. Moreover, preliminary evidence was obtained of a requirement for sensitivity in the TF donor.

The experiments reported here were designed to examine this system with particular regard to the

question of specificity. To this end, TF was prepared from donors of known sensitivity to tuberculin (PPD), streptococcal protein (SKSD) and diphtheria toxoid (DT). A preliminary report of the findings has been made (Salaman, 1976).

MATERIALS AND METHODS

Preparation of transfer factor (TF)

Healthy volunteers with strongly positive or negative Mantoux reactions were selected as blood donors for the preparation of TF. The positive group gave reactions at 48 h of at least 10 mm of induration in response to 0·2 μg of PPD (Evans Medical, Liverpool):—BW(01) gave 90/30 mm (erythema/induration); MC(02) 50/20; VW(03) 50/15; JM(04) 25/15; DD(05) 20/15; GR(06) 45/30; IA(08) 60/10. GS(07) and JD(09) were selected as positive donors on the basis of transformation response alone (Table 3). Negative reactors PC(51), AB(52), AT(53), MR(54), JW(55), GB(56) and AR(57) showed zero induration and no more than a trace of erythema at 2μg PPD.

The method used for preparation of TF was similar to that described by Ascher *et al* (1974). Venous blood (30 ml) containing 20 units/ml preservative-free heparin (Paines and Byrne, Greenford) and dextran (Sigma)—10% v/v of a 6% w/v solution in saline (mol. wt 254,000) was left to sediment for 35–45 min at 37°. The supernatant was removed and, after the white cells were counted, it was centrifuged at 200 *g* for 10 min at room temperature. Water (1·0 ml) was added to the white-cell pellet followed by 50 μg of DNase in 0·05 ml saline (Worthington, code DCL11S). The preparation was then put through ten cycles of freezing (acetone/dry ice) and thawing (25°). The resulting mixture was placed in boiled quarter-inch dialysis tubing (Scientific Instrument Centre, London) and dialysed overnight at 4° against 8–10 ml MEM-S containing antibiotic (see details of medium below). The dialysate was sterilised by filtration and used on the same day.

Action of TF on lymphocyte transformation

Blood donors for leucocyte cultures were Mantoux-negative as defined above or, though giving up to 6 mm erythema at 0·2 μg PPD, gave less than a 10-fold transformation response at 1 μg/ml PPD (donors JB(58), JR(59), MS(60) and RN(61)).

Leucocytes were obtained as above and resuspended at room temperature in MEM-S (Flow Laboratories) containing autologous heparinised plasma. Penicillin and streptomycin were included in MEM-S at 50 units/ml, and plasma was clarified by centrifugation at 1500 *g* for 15 min. Cultures were set up in quadruplicate in pyrex tubes (diameter 1 cm) as follows:—0·3 ml MEM-S/plasma containing $0{\cdot}5 \times 10^6$ mononuclear cells; up to 0·7 ml dialysate containing TF; 10 μl antigen as required; MEM-S to 1·0 ml. The final concentration of plasma was 15%.

The dialysate added to the cultures contained extract from $6{\cdot}5\text{–}8{\cdot}5 \times 10^6$ nucleated cells, the precise value being constant within each experiment. This quantity of TF is effective in enhancing transformation responses though it does not produce a maximal response (Ascher *et al.*, 1974). Mononuclear cells formed 25–45% of the nucleated cells from which these preparations were made: mean values were 32·2% (TF from tuberculin-positive donors) and 35·9% (negative donors).

PPD, a freeze-dried preparation of human tuberculin (Ministry of Agriculture, Weybridge) was dissolved in MEM-S. SKSD (streptokinase-streptodornase from Lederle) and DT (diphtheria toxoid from Wellcome) were dialysed exhaustively against phosphate-buffered saline and then diluted in MEM-S. Antigens were used in the cultures at final concentrations of 1μg/ml (PPD), 0·4, 2 or 4 streptokinase units/ml (SKSD) and 0·2 or 2 LF units/ml (DT).

Incubation was at 37° for 7 days in an atmosphere of 5% CO_2. A pulse of tritiated thymidine (TRA 120, Radiochemical Centre)—2·5 μCi at 50 mCi/mM —was given 18 h before harvest. At the time of the pulse, the cells were gently suspended by shaking the culture tubes. Cultures were filtered on glass-fibre discs which were washed with 10% TCA and methanol and placed in vials for scintillation counting.

Expression of results and statistical analysis

The values quoted of thymidine incorporation (counts/min) are geometric means from replicate cultures. These means were used in each experiment to obtain certain ratios which will be specified later. Geometric means of groups of such ratios have also been calculated.

To arrive at these various values, thymidine incorporation data were handled on a computer as their log transforms. The question whether a mean

ratio is significantly different from 1 or from another mean ratio was determined by Student's *t*-test on the log transformed data. Correlation coefficients (r) were similarly calculated on transformed data.

Assay of sensitivity of TF donors by lymphocyte transformation

Leucocytes were obtained as above and suspended at $0{\cdot}5 \times 10^6$ mononuclear cells/ml in RPMI 1640 (Flow Laboratories) containing antibiotic and 15% autologous plasma. Dilutions of antigens (10 μl) were added as required to 1·0 ml aliquots of the cell suspension, from which five replicate 0·18 ml cultures were set up in round-bottom plastic microplates (Linbro plates, Flow Laboratories). The cultures were incubated at 37° for 6 days in an atmosphere of 5% CO_2, and tritiated thymidine (0·5 μCi at 1 Ci/mM) was added 18 h before harvest. A Skatron automatic harvester with 10% TCA and methanol wash was used. Geometric means of thymidine incorporation in replicate cultures were calculated following scintillation counting, and sensitivity is expressed as the ratio of incorporation in the presence of antigen to that occurring in its absence (transformation index). Where sensitivity was determined on the same donor on separate occasions the geometric mean of the individual means was obtained (see Tables 3 and 4).

Antigens were used at the following final concentrations:—1 μg/ml (PPD), 4 streptokinase units/ml (SKSD) and 2 Lf units/ml (DT). These levels gave optimal discrimination in sensitivity between the various donors.

RESULTS

Effect of TF from tuberculin-positive and negative donors on transformation responses of lymphocytes from tuberculin-negative donors

The experiments carried out to determine the effect of TF on the transformation of lymphocytes are detailed in Table 1. From one to five TF preparations from tuberculin-positive or negative donors were tested in each experiment on lymphocytes from a tuberculin-negative donor. Cultures were for seven days in medium alone and in the presence of TF, PPD, SKSD, TF + PPD, and TF + SKSD. A pulse of tritiated thymidine was given over the final 18 h. PPD was used at 1 μg/ml. SKSD was used at 0·4, 2 or 4 units/ml to produce a response in the absence of TF of less than 10-fold, comparable to that occurring with PPD.

Figure 1 shows thymidine incorporation in these experiments in the presence of PPD plotted against that occurring in the presence of TF and PPD. TF from tuberculin-positive donors (Fig. 1a) usually raised the response over that seen with PPD alone (points lying above the nil-effect line). In many instances the effect was of considerable magnitude. On the other hand, 'negative' TF had little effect (Fig. 1b). The equivalent plots for SKSD are shown in Fig. 2. Again 'tuberculin-positive' TF was much more potent, although one strong stimulation was observed with 'negative' TF.

The results are analysed in Table 2. The column headed mean value of R(TF, PPD) gives the potency of TF from each donor expressed as the geometric mean of the ratios of thymidine incorporation in the presence of TF and PPD to that in the presence of PPD alone. TF from the same donor can give widely different ratios in different experiments, so that the value of potency can only be approximate when a particular donor was not tested repeatedly. Nevertheless, with one exception, mean values for tuberculin-positive donors were never less than 1·60, a value never achieved by negative donors. A similar situation obtained for SKSD although there was a high mean value of R(TF,SKSD) for one tuberculin-negative donor. The overall mean in the tuberculin system is the geometric mean of all values of R(TF,PPD) obtained with either 'positive' or 'negative' TF. The values are 1·89 and 1·15 respectively, and only the value for the positive group represents a significant stimulation ($P<0{\cdot}001$). The corresponding values for SKSD were 2·12 (significant stimulation, $P<0{\cdot}001$) and 1·25 ($P>0{\cdot}05$). Overall means for 'positive' and 'negative' TF were significantly different at the 2% level (PPD) and 1% level (SKSD).

So far no mention has been made of effects of TF in the absence of antigen. Mean values of R(TF)—the ratio of incorporation in the presence of TF to that in medium alone—are also given in Table 2. Overall means were 1·31 ('tuberculin-positive' TF) and 1·12 ('negative' TF). The former value represents a significant stimulation ($P<0{\cdot}05$), but the difference between 'positive' and 'negative' TF was not significant. This effect of TF is of course small in terms of absolute counts compared to effects obtained in the presence of antigen. Nevertheless, for 'positive' TF a significant correlation could be

Table 1. The effect of TF from tuberculin-positive and negative donors on the transformation of tuberculin-negative lymphocytes in the presence and absence of PPD and SKSD

Exp	Cells in culture	TF donor	Mean thymidine incorporation (c.p.m.)					
			No addition	TF	PPD	TF+PPD	SKSD	TF+SKSD
1	60	01	165	137	517	1099		
		53	165	222	517	573		
2	61	51	190	278	1347	271		
3	51	01	111	221	316	924		
		04	111	286	316	1196		
		52	111	314	316	576		
4	51	01	89	112	477	1912	285*	990
		53	89	91	477	721	285	804
5	51	05	357	95	533	1122	347*	676
		06	357	311	533	1052	347	767
		07	357	163	533	2360	347	1047
		53	357	101	533	729	347	183
6	53	01	62	86	258	299	78*	992
		08	62	80	258	162	78	111
		51	62	125	258	424	78	76
		52	62	56	258	234	78	210
7	51	01	95	133	138	117	952*	2706
		52	95	88	138	647	952	1108
8	52	01	83	161	29	119	203*	278
		07	83	189	29	193	203	708
		51	83	161	29	108	203	112
9	51	01	42	49	108	316	390*	1033
		52	42	55	108	132	390	240
10	51	01	89	187	249	336		
		07	89	109	249	633		
		51	89	55	249	137		
11	53	07	76	61	337	204	362‡	313
		51	76	55	337	569	362	702
12	52	01	192	312	417	801	335*	434
		07	192	253	417	397	335	444
		51	192	213	417	355	335	380
13	51	01	158	200	292	606	234‡	305
		03	158	236	292	488	234	292
		51	158	358	292	409	234	234
		52	158	287	292	317	234	234
14	53	01	98	109	461	365	299‡	657
		03	98	121	461	710	299	395
		51	98	96	461	414	299	360
		52	98	90	461	555	299	419
15	58	02	30	290	30	951	25‡	150
		09	30	151	30	102	25	177

Exp	Cells in culture	TF donor	Mean thymidine incorporation (c.p.m.)					
			No addition	TF	PPD	TF+PPD	SKSD	TF+SKSD
16	51	02	152	123	263	354		
		09	152	132	263	345		
		54	152	87	263	378		
17	59	01	113	139			158‡	199
		51	113	158			158	159
18	59	01	113	139			822*	1119
		51	113	158			822	1044
19	51	02	389	110	730	433	656†	1598
		53	389	68	730	734	656	3004
20	59	02	106	154	211	299	204†	792
		51	106	219	211	192	204	412
21	51	02	210	368	1018	1600	1089†	1832
22	59	01	104	175	756	633	321†	353
		02	104	147	756	934	321	367
		55	104	188	756	699	321	285
		56	104	150	756	533	321	401
		57	104	140	756	681	321	368

Cell and TF donors coded below 50 are tuberculin-positive; those coded above 50 are tuberculin-negative. PPD was used at 1μg/ml. SKSD concentration is shown for each experiment as follow: * 4 units/ml, † 2 units/ml, ‡ 0·4 units/ml. The concentration of TF and further details of the experimental procedure are given in Methods.

demonstrated between R(TF) and R(TF,PPD):— r = +0·54 ($P<0·001$). A similar tendency existed in the SKSD system though this was not significant (r = +0·30). It should be noted that no such correlations could be demonstrated using expressions based on increment in thymidine incorporation.

The effect of TF in the absence of PPD may be corrected out by expressing results in each test as R(TF,PPD) divided by R(TF). The overall mean for 'positive' TF was reduced by this procedure to 1·44 (representing significant stimulation, $P<0·02$) while that for 'negative' TF fell to 1·05. The corresponding figures for SKSD are respectively 1·62 ($P<0·01$) and 1·12. It was no longer possible to demonstrate significant differences between 'positive' and 'negative' TF.

Specificity of TF action on lymphocyte transformation

The results above showing a correlation between effects of TF from tuberculin-positive donors in the presence and absence of PPD suggest that the phenomenon is not antigen-specific, and this view is reinforced by the following considerations. Where available, the sensitivity of TF donors to PPD and SKSD is shown in Table 3 in terms of *in vitro* lymphocyte responsiveness. Whether expressed as transformation index or increment in incorporation on addition of antigen, there was as expected a significant correlation between PPD sensitivity and mean values of R(TF,PPD) or R(TF,SKSD); with transformation indices the values of r were respectively +0·79 ($P<0·01$) and +0·61 ($P<0·05$). By contrast, there was no correlation (r = −0·15) between SKSD sensitivity and R(TF,SKSD), demonstrating a lack of specificity in relation to SKSD and the independence of PPD and SKSD sensitivities. Moreover, not only was 'tuberculin-positive' TF generally more effective but the activity of individual preparations in the two antigen systems showed significant correlation (Fig. 3a; r = +0·43, $P<0·02$). No such link could be established for the smaller effects seen with 'negative' TF (Fig. 3b).

This analysis leaves out of account a possible small specific element obscured by non-specific

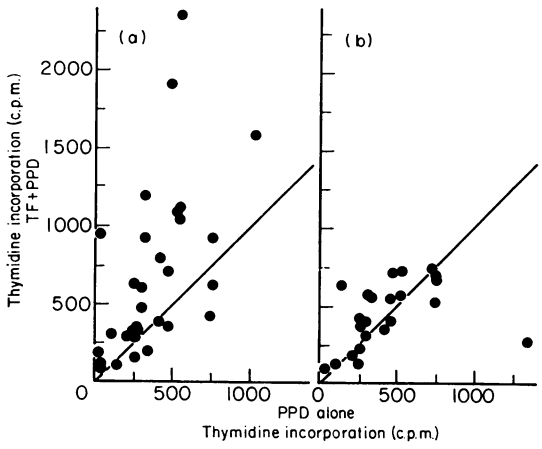

Figure 1. Effect of TF on the transformation of tuberculin-negative lymphocytes in the presence of PPD. Thymidine incorporation in the presence of PPD has been plotted against that occurring in the presence of TF+PPD. TF donors were tuberculin-positive (a) or tuberculin-negative (b). A line has been drawn through the origin with a gradient of 1.

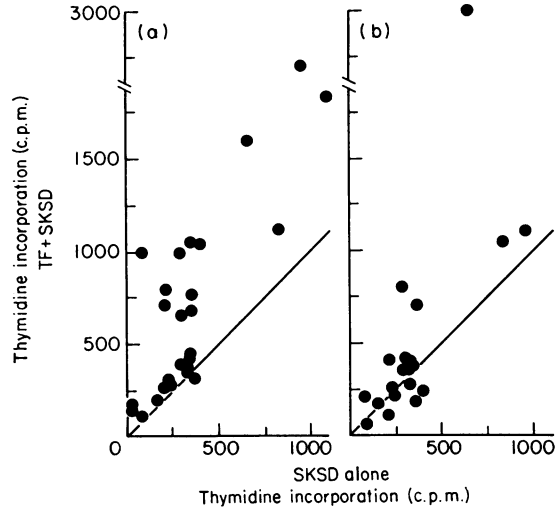

Figure 2. Effect of TF on the transformation of tuberculin-negative lymphocytes in the presence of SKSD. Thymidine incorporation in the presence of SKSD has been plotted against that occurring in the presence of TF+SKSD. TF donors were tuberculin-positive (a) or tuberculin-negative (b). A line has been drawn through the origin with a gradient of 1.

Table 2. Potency of TF from tuberculin-positive and negative donors

Tuberculin-positive TF donors	Mean transformation ratios			Tuberculin-negative TF donors	Mean transformation ratios		
	R(TF)	R(TF,PPD)	R(TF,SKSD)		R(TF)	R(TF,PPD)	R(TF,SKSD)
01	1·40(14;1·30)	1·78(12;1·82)	2·08(11;2·05)	51	1·34(11;1·55)	1·01(9;2·26)	1·15(9;1·48)
02	1·42(6;3·18)	2·03(6;4·04)	2·55(5;1·92)	52	1·32(6;1·59)	1·52(6;1·82)	1·22(5;1·71)
03	1·36(2;1·15)	1·60(2;1·06)	1·29(2;1·04)	53	0·51(4;2·68)	1·23(4;1·21)	1·90(3;3·11)
04	2·57(1)	3·79(1)	—	54	0·57(1)	1·44(1)	—
05	0·27(1)	2·10(1)	1·95(1)	55	1·80(1)	0·92(1)	0·89(1)
06	0·87(1)	1·97(1)	2·21(1)	56	1·44(1)	0·71(1)	1·25(1)
07	1·06(5;1·82)	2·12(5;2·74)	1·86(4;1·95)	57	1·34(1)	0·90(1)	1·14(1)
08	1·29(1)	0·63(1)	1·41(1)				
09	2·11(2;3·50)	2·12(2;1·97)	6·98(1)				
Overall means	1·31(33;1·96)	1·89(31;2·27)	2·12(26;1·91)		1·12(25;1·88)	1·15(23;1·84)	1·25(20;1·72)

Transformation ratios were calculated from the data in Table 1 as follows: R(TF) is the ratio of thymidine incorporation in the presence of TF to that occurring in medium alone. R(TF,PPD) is the ratio of incorporation in the presence of TF and PPD to that in the presence of PPD. R(TF,SKSD) is the ratio of incorporation in the presence of TF and SKSD to that in the presence of SKSD. The values shown for each donor are the geometric means of the transformation ratios obtained with their preparations. These are given with, in parentheses, the number of observations and the factor by which the mean is multiplied and divided to obtain the range of one standard deviation. For calculation of overall geometric means each individual test was placed in either the 'positive' or 'negative' group.

Table 3. Sensitivity of panel of TF donors to PPD and SKSD determined by transformation response

Donors	Baseline (c.p.m.)	Transformation index	
		PPD	SKSD
01	337(5;2·51)	50·1(5;1·56)	4·8(4;1·84)
02	79(2;1·44)	81·2(2;1·83)	40·9(2;1·44)
03	525(1)	15·5(1)	5·5(1)
07	183(1)	52·3(1)	—
09	217(1)	16·9(1)	2·5(1)
51	244(6;1·97)	2·8(6;1·87)	7·5(6;2·01)
52	276(3;1·69)	1·7(3;4·23)	2·0(2;1·26)
53	134(6;1·47)	10·5(6;1·82)	28·5(6;2·05)
54	254(1)	4·9(1)	1·2(1)
55	226(1)	2·2(1)	19·7(1)
56	147(1)	3·1(1)	3·6(1)
57	230(1)	1·5(1)	11·7(1)

Transformation responses of lymphocytes from TF donors were determined in microplates to antigens PPD (1 μg/ml) and SKSD (4 units/ml). Transformation index is the ratio of thymidine incorporation in the presence of antigen to that occurring in its absence. Geometric means of indices obtained in different experiments are shown with, in parentheses, the number of experiments and the factor by which the mean is multiplied and divided to obtain the range of one standard deviation. Baseline gives thymidine incorporation in the absence of antigen.

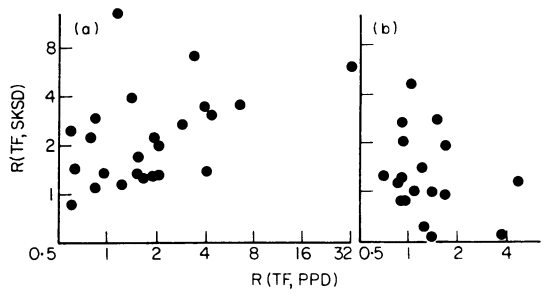

Figure 3. Relationship between the effectiveness of TF preparations in the PPD and SKSD transformation systems. Where preparations were tested in both systems, the corresponding values of the transformation ratios R(TF,PPD) and R(TF,SKSD) have been plotted against each other. Separate graphs are shown for TF from tuberculin-positive (a) and negative donors (b)

activity. This can best be investigated by examining the results with TF from tuberculin-negative donors, where activity expressed in the presence of PPD must presumably be non-specific. Thus, the ratio of R(TF,SKSD) to R(TF,PPD) could represent SKSD-specific activity. However, the overall mean of this ratio for 'negative' TF was less than 1 (0·99) and there was no significant correlation between SKSD sensitivity and the mean of these ratios for individual donors ($r = +0·20$). A similar result is obtained by computing the relationship between SKSD sensitivity and the ratio of R(TF,SKSD) to R(TF) throughout both donor groups ($r = +0·28$). Clearly, any element of specificity present must be at an extremely low level. Negative donor 53 coupled high SKSD sensitivity with a high value of R(TF,SKSD); it is of interest, therefore, that her sensitivity to PPD *in vitro* was higher than expected in a Mantoux-negative subject.

Further evidence for lack of antigen specificity was provided in experiments in which diphtheria toxoid (DT) was used as antigen (Table 4). One of the TF donors (09) gave a massive delayed skin reaction to a Schick-test dose of DT (65 mm induration), and was highly sensitive in transformation as shown. Donor 51 was not skin-tested but showed a moderate transformation response. Other donors were not sensitive in transformation. In none of the experiments did either of these sensitive donors produce the largest value of R(TF,DT). Indeed, this ratio appeared to reflect tuberculin sensitivity as in the previous experiments.

Other relationships in the transformation system

Other workers using systems similar to that described here have found that the augmentation effect of TF (expressed as the increment in thymidine incorporation on addition of antigen in the presence of TF less the corresponding computation in the absence of TF) increases in relation to the antigen responsiveness of the cultured lymphocytes (expressed as in the second part of the above expression) (see Hamblin, Maini & Dumonde, 1976). This was not, however, a pronounced feature of the present data, and only for 'tuberculin-positive' TF in the SKSD system was there a correlation between these two parameters ($r = +0·42$, $P<0·02$). Moreover, any such relationships disappear when effects of TF are expressed in ratios as in this paper.

If TF preparations contain antigen, a correlation between the effect of antigen in the absence of TF and that of TF in the absence of antigen might be expected. Such an analysis has been carried out for the tuberculin system in which the concentration of antigen was not varied. No correlation was found between R(TF) and R(TF,PPD): the values of r for

Table 4. Effect of TF on transformation response to diphtheria toxoid (DT): significance of DT sensitivity of TF donor

Exp	Cells in culture	TF donor	Mean thymidine incorporation (c.p.m.)				Transformation ratio	Sensitivity of TF donor to DT
			No addition	TF	DT	TF+DT	R(TF,DT)	
12	52	01	192	312	176	373	2·12	1·4(3;2·70)
		07	192	253	176	318	1·81	—
		51	192	213	176	249	1·42	9·0(4;1·94)
16	51	02	152	123	425	1113	2·62	1·3(2;1·39)
		09	152	132	425	283	0·67	30·7(1)
		54	152	87	425	404	0·95	1·1(1)
23	58	02	95	68	446	1000	2 24	1·3(2;1·39)
		09	95	100	446	867	1·94	30·7(1)
		51	95	66	446	645	1·45	9·0(4;1·94)

R(TF,DT) is the ratio of thymidine incorporation in the presence of TF and DT to that occurring in the presence of DT alone. DT was used at 2 (Exp 12, 23) or 0·2 Lf units/ml (Exp 16). Sensitivity of TF donors was determined as in Table 3 with DT at 2 Lf units/ml, and it is expressed as transformation index.

'positive' and 'negative' TF are respectively —0·17 and +0·11. The same conclusion is reached if effects are expressed as augmentation of thymidine incorporation.

DISCUSSION

These experiments provide no support for the concept of antigen-specificity in TF. Firstly, tuberculin-sensitivity of TF donor was a marker for the effectiveness of TF in enhancement of transformation not only for PPD but also for SKSD and DT. Sensitivity of TF donors to SKSD and DT was not a determining factor in potency of TF. Secondly, TF from tuberculin-negative donors occasionally showed a moderate level of activity in the tuberculin system. This cannot be due to a tuberculin-specific factor so that a non-specific activity is undoubtedly present. Thirdly, TF frequently increased the background level of transformation occurring in the absence of antigen. Although a small effect in terms of absolute counts, this ability tended to parallel the activity of TF in the presence of antigen. Finally, analysis of the results failed to reveal a specific factor operating in the face of a more potent non-specific activity.

There are now several other reports on the effects of TF on antigen-induced lymphocyte transformation and in none of these is there convincing evidence of specificity (Arala-Chaves, Ramos & Porto, 1976; Ascher & Andron, 1976; Erickson, Holzman, Valentine & Lawrence, 1976; Hamblin *et al.*, 1976; Littman, Hirschman & David, 1977). Experiments in which 'cell suicide' techniques were employed to eliminate antigen-sensitive cells from the system suggest that no transfer of sensitivity to uncommitted cells occurs (Burger, Vandenbark, Finke, Nolte & Vetto, 1976; Cohen, Holzman, Valentine & Lawrence, 1976). The picture that emerges from these reports and from the present study is of a non-specific factor which stimulates DNA synthesis without having significant mitogenic activity in its own right. This action may involve an increase in the rate of DNA synthesis and/or in the number of cells participating, either precommitted cells or cells recruited by blastogenic factor. The effect of TF on transformation in the absence of added antigen may be dependent on undefined antigen in the system or on ongoing DNA synthesis in the cells. It is not likely to be mitogenic in view of the evidence (see above) suggesting that the same stimulatory factor is involved in both the presence and the absence of antigen. Less consistent results have been obtained for stimulation by TF of mitogen-induced transformation (Hamblin, Dumonde & Maini, 1976; Burger *et al.*, 1976). The

significance of this is not clear though it may relate to my own experience of unpredictable TF effects when response to antigen alone is strong (over 10-fold).

The nature of the factor responsible for this effect is not known. However, it is of interest that the synthetic double-stranded polynucleotide, poly A:U, behaves in a similar manner in its effects on background and on antigen and mitogen-induced transformation (Chess, Levy, Schmukler, Smith & Mardiney, 1972). From kinetic studies it was concluded that poly A:U acts by increasing the number of cells participating in the response. Likewise, we do not know which cell types in the buffy-coat preparation are a source of the factor, other than that activity has been reported in preparations from purified polymorphs (Littman *et al.*, 1977).

This study differs from other reports in that it demonstrates a relationship between TF activity and donor sensitivity, albeit a relationship which does not involve antigen-specificity. The finding that TF from tuberculin-positive donors was in general more potent than that from negative donors mimics an earlier study, with a different panel of donors, on the effect of TF on the migration of normal guinea-pig peritoneal exudate cells in the presence and absence of PPD (Salaman, 1974). It seems reasonable to suppose therefore that the same non-specific factor is involved. The principal effect observed was inhibition of migration by PPD in the presence of 'positive' TF, and this can be seen as enhancement by TF of low-level, environmentally-acquired responsiveness to PPD. On the other hand, in studies with antigens DT and haemocyanin it has not been possible to exclude an element of TF specificity in the migration system (unpublished work of Sargent, Zanelli & Salaman).

What is the explanation of the greater potency of the preparations from tuberculin-positive donors? There is no obvious way in which components of *M. tuberculosis* or BCG possessing adjuvant activity could be implicated. Moreover, tuberculin-sensitivity as such seems unlikely to be important. On the other hand, the ability to express strong delayed reactions could be, though it is not possible to tell from the data whether strength of reaction or simply a positive reaction is the important criterion. Indeed, it may be the tuberculin-negative group that should claim our attention. Although it was not possible to obtain reliable information on this point, some members of this group may have had BCG but failed to develop persistent sensitivity. The factor responsible for enhancing DNA synthesis could be a discrete low molecular weight molecule present in the cells, or it may represent breakdown products of cellular macromolecules that are formed during the course of preparation. If the latter, a higher level of hydrolytic enzymes in the 'positive' cells could explain the difference between the donor groups.

There have been two other studies in which a systematic comparison of TF from tuberculin-positive and negative donors has been made, and it is not clear why the same phenomenon was not evident. Hamblin *et al.* (1976) used a culture system similar to that used here. In many of their experiments antigen alone produced a stimulation of more than 10-fold, and there was a tendency for 'positive' TF to be more potent than 'negative' TF when the effect of antigen was small (see their Fig. 2). On the other hand, 'negative' TF was consistently more potent than 'positive' TF when all experiments in which TF itself stimulated incorporation are considered (see their Fig. 3c). In selection of negative donors a very low level of induration was permitted. Erickson *et al.* (1976) do not state their criterion for negativity. They used a microplate culture system, and many of their 'positive' preparations were tested on cells responding strongly to antigen.

The parallel between TF and synthetic polynucleotide mentioned above in relation to their effects on transformation can be extended. Polynucleotides have been shown to initiate the maturation of T lymphocytes (Scheid, Hoffman, Komuro, Hammerling, Abbott, Boyse, Cohen, Hooper, Schulof & Goldstein, 1973) and to act as adjuvants for T cell responses *in vivo* (Cone & Johnson, 1972; Casavant & Youmans, 1975). This first point recalls the ability of TF to increase PHA responsiveness and T cell rosettes in immunodeficient patients (Wybran, Levin, Spitler & Fudenberg, 1973; Griscelli, Revillard, Betuel, Herzog & Touraine, 1973; Valdimarsson, Hambleton, Henry & McConnell, 1974), and direct evidence of TF as an adjuvant for delayed responses has been obtained in the guinea-pig (Wilson, Welch & Fudenberg, 1977). This does not prove that the active principal in TF is polynucleotide. However, if this range of activities can be brought about by a single chemical species (i.e. poly A:U) considerable weight is added to the hypothesis that the non-specific factor in leucocyte

dialysates responsible for the enhancement of transformation is involved in the 'transfer' of sensitivity *in vivo*. Further, in view of the absence of convincing evidence of specificity in man and in model systems the factor active in transformation could conceivably be responsible for the entire TF phenomenon. In the light of these arguments, tuberculin-sensitivity of TF donor may be a useful marker for preparations best effective in restoring cell-mediated immunity in patients.

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