Cell-mediated Immunity in Diabetes Mellitus

Lymphocyte Transformation by Insulin and Insulin Fragments in Insulin-treated and Newly-diagnosed Diabetics

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SUMMARY

Using a radioisotope labeling technic, the ability of bovine and porcine insulin antigens to induce lymphocyte transformation was tested with cells from the peripheral blood of thirty nondiabetic controls, fifty established insulin-dependent diabetics with no evidence of insulin allergy, and ten newly diagnosed diabetics (five untreated, five insulin-treated for less than three weeks). Lymphocytes from twenty-six (42 per cent) of the diabetics showed significant blastogenesis to bovine or porcine insulin, as compared with two (7 per cent) of controls; the phenomenon was shown by both established and newly diagnosed patients including four who had never received insulin. The results indicate that cellular hypersensitivity to insulin, as judged by an in vitro test, is relatively common in insulin-treated diabetics without in vivo evidence of allergy, and suggest that hypersensitivity may also be present in untreated diabetics.

Lymphocytes from twenty-one of the twenty-six diabetics who responded to intact insulin were further tested using bovine and porcine insulin A chain and bovine B chain as antigens. The A chain of either insulin induced significant blastogenesis in only one diabetic but bovine B chain induced significant blastogenesis in fourteen (67 per cent) of the patients tested. These results suggest that B chain is the major antigenic site determining cellular hypersensitivity to insulin. DIABETES *24*:36-43, January, 1975.

Cell-mediated immune mechanisms in human diabetes mellitus have recently been studied by in vitro tests. The leucocyte migration test[1] has demonstrated migration inhibition of diabetic leucocytes cultured in the presence of antigens derived from porcine,[2] fetal calf[3] or human pancreas.[4] The phenomenon was most common in recently-diagnosed, juvenile-onset diabetics and was independent of antidiabetic treatment, being found in patients who were insulin-dependent, insulin-independent or untreated at the time of study.[2-4] The same investigators did not find migration inhibition of diabetic leucocytes by antigens derived from liver, kidney, thymus or adrenal, nor did purified insulin inhibit migration; it has therefore been suggested that the results indicated the presence in young diabetics of a state of cellular immunity to species-nonspecific antigen(s), pancreatic in origin but different from insulin. The organ-specificity of the antigen must, however, remain in doubt at present in view of reports[5,6] that liver mitochondria can inhibit migration of leucocytes in diabetes as well as in other diseases[7-9] of established autoimmune etiology.

Mitogen- or antigen-induced transformation of lymphocytes to blast cells[10] are alternative in vitro tests of cellular immune function. The mitogen phytohemagglutinin (PHA) is considered to stimulate mainly T lymphocytes[10,11] and has been used to demonstrate that transformation responses are normal in well controlled diabetics[12,14] and depressed in poorly controlled diabetics.[13,14] In contrast there is little information concerning antigen-induced lymphocyte transformation in diabetes, but various investigators[15-17] have shown that bovine insulin can induce blastogenesis in lymphocytes from diabetics with both immediate (urticarial) and delayed (cutaneous) insulin allergy.

In the present investigation we have used bovine and porcine insulin as antigens to detect sensitized lymphocyte populations in treated diabetics without in vivo evidence of insulin allergy, and in untreated diabetics; in addition, isolated fragments (A and B

TABLE 1

Composition of Lilly bovine and porcine insulins used as antigens

Content*	Bovine Insulin	Porcine Insulin
Proinsulin	0.06	0.15
Glucagon	0.007	0.002
Zinc	0.80	0.70
Monodesamido insulin	13.0	7.0
"Arginine" insulins	1.0	2.0
Potency (U/mg.)	25.4	25.4

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Accepted for publication October 1, 1974.

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chain) of the insulins from both species have been used in an attempt to localize the immunogenic portion of the insulin molecule.

PATIENTS AND METHODS

Patients. Sixty diabetics were studied. Forty-two were women and eighteen were men, from sixteen to seventy-one years of age (mean 42.1). Fifty were established diabetics and have been insulin-treated between six months and twenty-four years. Ten were newly diagnosed diabetics, five of whom had been insulin-treated for three weeks or less and five of whom were untreated at the time of study. All were attending an outpatient clinic and in all (except for the newly-diagnosed) the disorder was well controlled as judged by the following criteria: steady weight and insulin dosage; absence of thirst or polyuria; no heavy glycosuria; no ketonuria; mid-morning blood glucose less than 250 mg. per 100 ml. All were taking insulin of bovine origin, prepared either as soluble (Regular) insulin or in a sustained-release form (Protamine-Zinc or IZS Lente insulin). None had evidence of immediate or delayed-type insulin allergy.

TABLE 2

Ability of varying concentrations of bovine insulin to induce transformation (judged by ^{3}H-thymidine uptake) of cultured lymphocytes from a diabetic with insulin allergy. No significant increase of blastogenesis is obtained by adding more than 10 μg insulin per culture.

	Control* Cultures	Cultures plus Bovine Insulin Antigen* 50 μg/ml. 1 μg/culture	500 μg/ml. 10 μg/culture	1,000 μg/ml. 20 μg/culture	5,000 μg/ml. 100 μg/culture
First Assay	1,080	8,720	12,480	13,640	10,390
Second Assay	1,769	9,550	14,570	14,210	12,620

*All results as cpm

The control group comprised thirty healthy non-diabetic volunteers. For statistical purposes it was selected to be comparable in terms of sex (twenty women, ten men) and age (range fifteen to seventy-two years, mean 40.8 years) with the diabetic group. Most controls were laboratory or hospital personnel; a few were hospital outpatients who were not known to

TABLE 3

Ability of bovine insulin, porcine insulin and PHA to induce transformation (judged by ^{3}H-thymidine uptake) of cultured lymphocytes from thirty normal subjects. Results expressed both as counts per minute (cpm) and transformation index (T.I.—see text). All figures are means of triplicate cultures.

Age/sex	Control	Bovine Insulin		Porcine Insulin		Maximal PHA
	cpm	cpm	T.I.	cpm	T.I.	Response (cpm)
15/m	939	546	0.58	751	0.80	25,100
72/f	1,318	806	0.61	1,216	0.92	17,900
29/f	704	501	0.71	613	0.87	44,400
19/m	1,188	893	0.75	1,463	1.23	22,800
34/f	1,026	784	0.76	1,281	1.24	15,000
34/f	823	669	0.80	707	0.86	16,300
66/f	975	792	0.81	800	0.82	8,700
32/f	1,133	941	0.83	1,497	1.32	22,500
53/m	1,572	1,322	0.85	1,352	0.86	38,400
24/f	759	654	0.86	502	0.66	16,800
58/f	1,033	914	0.88	1,109	1.07	11,100
36/m	1,007	899	0.89	1,025	1.02	14,900
26/f	659	589	0.90	594	0.90	26,100
68/f	1,583	1,521	0.96	1,456	0.92	21,200
32/m	1,292	1,250	0.97	1,066	0.83	10,800
48/f	875	860	0.98	684	0.78	19,800
41/f	958	940	0.98	937	0.98	19,200
28/f	1,106	1,104	1.00	1,175	1.06	44,700
36/f	1,136	1,178	1.04	1,264	1.11	14,100
18/m	848	891	1.05	903	1.06	29,300
69/m	710	750	1.05	726	1.02	22,500
54/f	1,285	1,363	1.06	926	0.72	7,600
26/f	955	1,042	1.09	1,781	1.86	20,300
46/f	797	880	1.10	1,260	1.58	27,400
48/f	923	1,017	1.10	991	1.07	13,300
45/m	1,714	1,910	1.11	1,980	1.16	23,500
32/f	1,073	1,222	1.14	1,300	1.21	38,500
57/m	1,809	2,583	1.43	1,835	1.01	16,700
36/m	1,465	2,109	1.44	1,584	1.08	30,000
48/f	968	1,457	1.51	1,327	1.37	54,100

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TABLE 4

Ability of bovine insulin, porcine insulin and PHA to induce transformation (judged by ^{3}H-thymidine uptake) of cultured lymphocytes from fifty established insulin-dependent diabetics. Results as in table 3.

Study	Age/Sex	Control	Bovine Insulin		Porcine Insulin		Maximal PHA
Number		cpm	cpm	T.I.	cpm	T.I.	Response (cpm)
1	27/m	964	705	0.73	1,039	1.08	35,900
2	50/f	1,541	1,123	0.73	1,326	0.86	17,500
3	42/f	1,495	1,146	0.85	1,053	0.80	22,400
4	40/f	809	728	0.90	802	0.98	18,700
5	31/m	1,475	1,388	0.94	1,400	0.95	26,400
6	62/f	878	843	0.96	805	0.92	24,100
7	65/f	1,096	1,071	0.98	956	0.87	30,700
8	40/f	1,167	1,172	1.00	1,006	0.86	17,600
9	59/f	1,122	1,120	1.00	1,327	1.10	20,400
10	66/f	994	1,001	1.01	1,351	1.36	18,500
11	45/m	1,226	1,270	1.04	1,164	0.95	26,400
12	38/f	915	959	1.05	1,049	1.15	25,000
13	42/m	1,518	1,597	1.05	2,173	1.43	13,900
14	25/f	728	760	1.05	881	1.21	17,400
15	48/f	1306	1,427	1.09	1,259	0.96	7,800
16	46/f	811	900	1.11	853	1.05	41,800
17	55/f	963	1,079	1.12	1,014	1.05	21,400
18	20/f	870	979	1.13	1,357	1.56	33,300
19	37/f	931	1,051	1.13	863	0.93	27,600
20	69/m	1,071	1210	1.13	1,089	1.02	10,600
21	23/f	1,000	1,140	1.14	1,320	1.32	19,300
22	46/m	939	1,067	1.14	1,221	1.30	27,400
23	41/f	1043	1,210	1.16	1,079	1.03	12,700
24	45/f	720	848	1.18	794	1.10	15,500
25	21/f	923	1,155	1.25	1,609	1.74	50,600
26	70/f	1,320	1,690	1.28	1,990	1.51	20,400
27	25/f	1,448	1,853	1.28	1,445	1.00	26,600
28	62/m	962	1,228	1.28	1,062	1.11	38,200
29	14/f	634	818	1.29	799	1.26	8,500
30	27/f	859	1,112	1.30	1,120	1.30	11,900
31	22/m	951	1,109	1.31	1,561	1.83	21,100
32	67/f	869	1,169	1.34	927	1.07	21,200
33	57/m	1,446	1,952	1.35	2,053	1.42	39,500
34	52/f	1,094	1,557	1.42	1,503	1.37	14,000
35	46/m	832	1,192	1.43	1,278	1.53	10,300
36	42/f	1,391	2,130	1.53	2,465	1.77	18,200
37	30/f	989	1,533	1.55	1,553	1.57	35,900
38	41/f	943	1,525	1.62	1,719	1.82	19,200
39	37/f	885	1,430	1.62	1,686	1.90	27,900
40	48/f	1,000	1,659	1.66	1,809	1.81	26,700
41	61/f	1,832	3,077	1.68	2,994	1.63	18,900
42	54/f	1,016	1,802	1.77	1,641	1.61	20,500
43	59/f	1,547	2,769	1.79	2,614	1.69	14,100
44	52/f	843	1,512	1.79	2,366	2.80	17,600
45	61/m	1,042	1,963	1.88	1,777	1.71	26,000
46	62/f	897	1,790	1.99	1,282	1.42	47,100
47	26/f	734	1,508	2.06	1,454	1.98	21,300
48	71/f	1,458	3,164	2.17	3,762	2.58	41,000
49	38/m	864	2,449	2.83	2,049	2.38	13,900
50	39/f	1,046	3,797	3.63	4,707	4.50	34,500

have endocrine or immunological disease.

Antigens. Bovine and porcine insulins of known potency and composition (table 1) were obtained from the Lilly Research Laboratories. Bovine insulin A chain and B chain (aminoethylated), and porcine insulin A chain (S-sulfonate) were obtained from the same source. All preparations were in crystalline powder form. On the day of use they were dissolved in a mixture of 10 per cent sterile phosphate buffered saline/80 per cent Eagle's Basal Medium/10 per cent fetal calf serum.

The optimal concentration of insulin for use in our investigations was determined by measuring the blastogenic effect of insulin on lymphocytes from a diabetic with known insulin allergy; the patient was a twenty-two-year-old man who had a daily insulin re-

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TABLE 5

Ability of bovine insulin, porcine insulin and PHA to induce transformation (judged by ^{3}H-thymidine uptake) of cultured lymphocytes from ten newly diagnosed diabetics. Results as in table 3.

Study Number	Age/Sex	Control cpm	Bovine Insulin cpm	Bovine Insulin T.I.	Porcine Insulin cpm	Porcine Insulin T.I.	Maximal PHA Response (cpm)
51	58/f	1,505	1,472	0.98	1,430	0.95	17,700
52	37/m	841	832	0.99	699	0.83	7,800
53	19/m	1,043	1,029	0.99	1,343	1.29	19,400
54	42/m	891	972	1.09	881	0.99	9,900
55	18/m	619	680	1.10	930	1.50	13,800
56	19/f	1,473	2,458	1.67	2,591	1.76	38,000
57	23/f	757	1,498	1.98	1,520	2.00	25,400
58	30/m	802	1,626	2.03	1,729	2.16	16,500
59	16/m	1,138	2,675	2.35	2,281	2.00	25,400
60	19/f	1,307	5,313	4.07	5,925	4.53	16,600

quirement in excess of 200 units in addition to cutaneous reactions to bovine insulin injections, and who therefore showed evidence of both humoral and cell-mediated insulin hypersensitivity. Lymphocytes were obtained from this patient on two separate occasions, prepared and cultured as described below, and exposed to varying concentrations of bovine insulin antigen. The results (table 2) show that maximal blastogenesis was stimulated with insulin at a concentration of 500 μg/ml. (10 μg/culture), no significant increase in lymphocyte ^{3}H-thymidine uptake being induced by higher concentrations of insulin. Thus on the basis of this preliminary dose-response analysis, all experiments were performed with solutions containing 500 μg/ml. intact insulin or insulin fragment.

Sample Collection and Lymphocyte Culture. Fifteen milliliters venous blood was removed from each patient and control at mid-morning and anticoagulated with preservative-free heparin. Lymphocytes were prepared from whole blood by the methods (density centrifugation, repeated washing in EBM) described in previous studies[14,18] from this laboratory, and suspended in EBM with 10 per cent fetal calf serum at a concentration of 1×10^6 cells per milliliter.

TABLE 6

Effects of bovine and porcine insulin on PHA-induced transformation of lymphocytes from five normal subjects. Neither insulin has a significant effect on the ^{3}H-thymidine uptake of PHA-stimulated lymphocytes.

Age/Sex	Maximal ^{3}H-thymidine Uptake (cpm) With PHA	PHA+B.I.*	PHA+P.I.*
36/m	32,000	35,000	31,000
19/m	25,000	24,000	22,000
32/m	9,000	8,000	9,000
26/f	28,000	26,000	26,000
36/m	14,000	16,000	14,000

*B.I.—Bovine insulin 10 μg/culture
P.I.—Porcine insulin 10 μg/culture

A portion of the lymphocytes from each subject was cultured in the presence of the mitogen phytohemagglutinin (PHA) to provide a convenient assessment of the adequacy of general cell-mediated immune func-

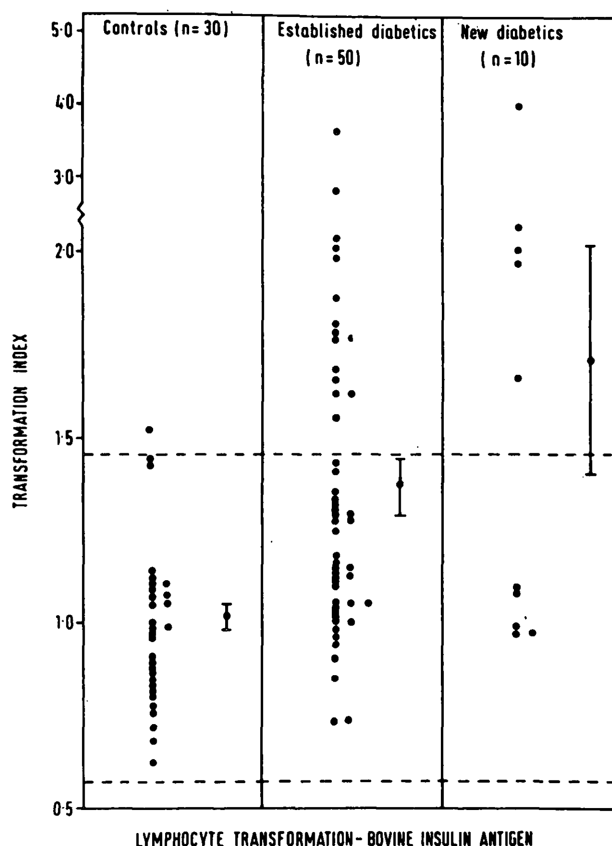

FIG. 1. Lymphocyte transformation in controls and diabetics induced by bovine insulin antigen. The mean transformation index ± S.E.M. is shown to the right of the individual results for each group. Dotted lines (- - - -) indicate the normal range (mean ± 2 S.D.) of transformation indices in control subjects. Significant transformation (index >1.45) is shown by fifteen established and five newly diagnosed diabetics as compared to one control. Both diabetic groups differ significantly ($p < 0.01$) from the control group.

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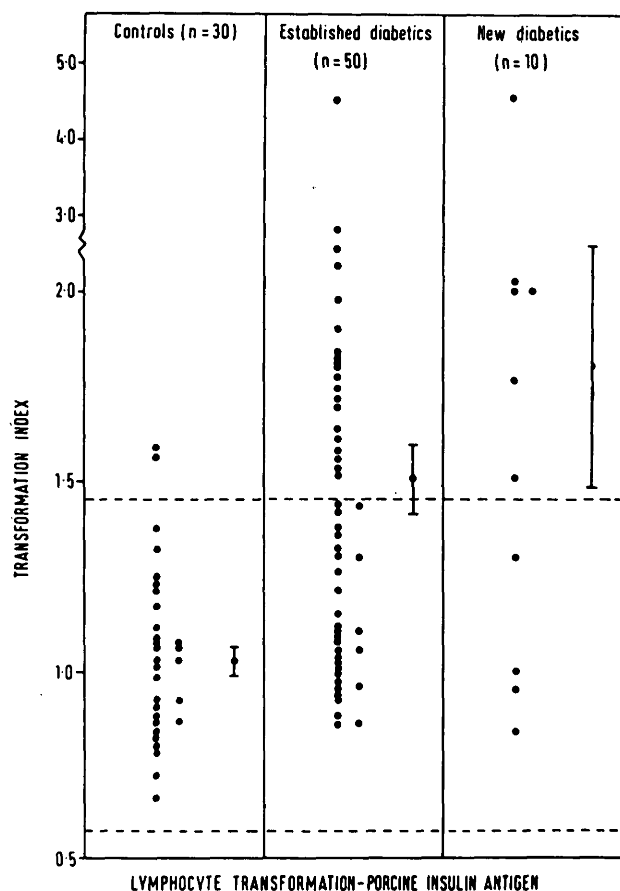

FIG. 2. Lymphocyte transformation in controls and diabetics induced by porcine insulin antigen. Symbols as in figure 1. Significant transformation is shown by twenty established and six newly diagnosed diabetics as compared to two controls. Both diabetic groups differ significantly ($p < 0.01$) from the control group.

tion. The lymphocyte transformation response to PHA was measured by an extensively investigated micromethod,[19] which utilizes the cellular uptake of radioactive DNA precursor (^{3}H-thymidine) as the index of lymphocyte stimulation and has been used in our previous study[14] of PHA responses in diabetes mellitus. This micromethod enables a mitogen dose-response curve to be constructed for lymphocytes from each subject by using PHA at concentrations of 0.32, 0.63 and 1.25 μl per milliliter culture. The radioactivity of stimulated cells was determined by an automatic beta counter (Nuclear Enterprises NE8312) and results are expressed as counts per minute (cpm).

The remaining lymphocytes from each patient and control were cultured in the presence of insulin antigen. The technic used was modified from the PHA micromethod[19] and is briefly summarized as follows: aliquots of 2×10^5 lymphocytes were pipetted into the wells of plastic tissue culture plates (Cooke Microtiter). All experiments were performed in triplicate and consisted of a control row of cultures without antigen, and rows to which 20 μl of the required antigen solution (i.e. 10 μg insulin or insulin fragment) was added. The plates were gassed with a 95 per cent air/5 per cent CO_2 atmosphere and incubated at 37°C. in sealed, humidified containers. After five days the cultures were labeled with ^{3}H-thymidine (specific activity 5 Ci/mmol, dose 0.4 μCi per culture) and terminated sixteen hours later by harvesting the cells onto glassfibre filter discs and preparing them[19] for scintillation counting. The prepared samples were counted in an automatic beta counter.

Results were obtained as counts per minute (cpm); the effect of antigen in stimulating blastogenesis (the 'transformation index') was expressed by the formula:

$$\text{Transformation Index} = \frac{\text{cpm of cultures with antigen}}{\text{cpm of cultures without antigen}}$$

The normal range of transformation indices using intact insulin antigens was obtained from the mean transformation index ± 2 standard deviations of the results given by both insulins in the control group (i.e. 60 experiments). The range in normals by this method was found to be 0.57-1.45 (mean 1.01 $\pm$ 0.44), and a transformation index above 1.45 was thus taken to indicate significant antigenic stimulation of blastogenesis. Several cultures from controls and diabetics were examined by light microscopy to confirm that the uptake of radioactive label was paralleled by morphological blast transformation.

All diabetics and controls were tested with intact bovine and porcine insulin antigens. Lymphocytes from those patients who showed significant transformation were further tested with bovine A and B chain and porcine A chain.

RESULTS

Mitogenic effects of PHA. The maximal PHA response of diabetics and controls (i.e. the highest lymphocyte incorporation of ^{3}H-thymidine label induced by one of the three concentrations of PHA used) is shown in tables 3-5. The mean maximal PHA response in the thirty controls was 23.1 $\pm$ 4.3 (expressed as cpm $\times 10^3 \pm$ S.E.M.) while the corresponding figure in the sixty diabetics was 22.5 $\pm$ 4.7. The two groups do not differ significantly in PHA responsiveness and the results confirm our earlier observation[14] that mitogen-induced lymphocyte transformation is normal in diabetes mellitus, except at times of severe metabolic decompensation.

The maximal PHA response of five control subjects was further measured in lymphocyte cultures to which

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TABLE 7

Ability of bovine insulin A chain, porcine insulin A chain and bovine B chain to induce transformation (judged by ^{3}H-thymidine uptake) of cultured lymphocytes from twenty-one diabetics who showed significant transformation to intact insulin molecule. 'Study numbers' identify patients in tables 4 and 5. Results as in table 3.

Study	Control	Bovine A Chain		Porcine A Chain		Bovine B Chain	
Number	cpm	cpm	T.I.	cpm	T.I.	cpm	T.I.
49	970	942	0.97	739	0.76	680	0.70
47	1,004	1,104	1.10	1,670	1.69	944	0.94
38	1,150	1,220	1.06	781	0.68	1,380	1.20
*58	902	939	1.04	747	0.83	1,136	1.26
41	1,623	1,445	0.89	—	—	2,173	1.34
45	1,684	1,161	0.69	—	—	2,339	1.39
36	1,092	963	0.88	—	—	1,527	1.40
40	870	938	1.08	523	0.60	1,313	1.51
*60	1,422	1,466	1.03	—	—	2,415	1.70
44	759	639	0.84	834	1.10	1,364	1.80
43	1,670	1,385	0.83	—	—	3,173	1.90
*57	518	682	1.10	—	—	1,171	1.90
42	925	1,833	1.98	906	0.98	1,880	2.03
*59	1,062	1,010	0.95	1,094	1.03	2,123	2.10
26	1,566	1,598	1.02	1,690	1.08	3,445	2.20
*56	1,241	1,450	1.17	—	—	6,452	5.20
39	950	1,020	1.07	—	—	5,605	5.90
37	860	1,039	1.20	—	—	6,192	7.20
48	1,287	1,364	1.06	1,094	0.85	11,866	8.70
25	705	750	1.05	—	—	6,201	8.80
46	964	972	1.01	630	0.66	9,350	9.70

*Newly diagnosed patient

10 μg bovine or porcine insulin had been added. The results (table 6) indicate that neither bovine nor porcine insulin have an appreciable effect on ^{3}H-thymidine uptake in the presence of PHA when added to forty-eight-hour cultures in the stated concentrations.

Antigenic effects of intact insulins. The effects of bovine and porcine insulin antigens in inducing blastogenesis of lymphocytes from controls and diabetics are presented in tables 3-5 (as absolute cpm) and in figures 1 and 2. Only one normal subject showed significant transformation using bovine insulin antigen; in contrast fifteen established and five newly diagnosed diabetics (three untreated, two insulin-treated for less than three weeks) showed significant transformation, their indices ranging from 1.53 to 4.07 (figure 1). The groups of both established and newly diagnosed diabetics differed significantly from the control group ($p < 0.01$ by Wilcoxon's test).

Experiments using porcine insulin as antigen (figure 2) gave a virtually identical pattern. Two control subjects showed significant transformation, as compared with twenty established and six newly diagnosed diabetics (four untreated, two treated for less than three weeks). Both diabetic groups again differed significantly ($p<0.01$) from the controls. Almost all the diabetics who showed transformation to porcine insulin also showed transformation to bovine insulin (tables 4 and 5).

Antigenic effects of A and B chains. Lymphocytes were available for study from twenty-one of the twenty-six diabetics who showed significant transformation to intact insulin molecule. The effects of bovine A chain, porcine A chain and bovine B chain on these lymphocytes are shown in table 7 and figure 3. The A chain of either insulin was almost without effect, inducing significant blastogenesis in only one diabetic; in contrast the B chain of bovine insulin induced significant lymphocyte transformation in fourteen (67 per cent) of the twenty-one patients tested. In some diabetics the isolated B chain appeared to be more potent in stimulating blastogenesis than intact insulin molecule, the transformation index being greater than 4.0 in six patients. Statistical analysis confirmed that the effects of B chain on lymphocyte transformation differed significantly ($p<0.01$) from those of the A chain of either insulin.

DISCUSSION

Under the stated culture conditions, intact insulin (bovine and porcine) was observed to induce transformation of lymphocytes in vitro from one-third of the diabetics tested. In this context, insulin may be regarded as showing cellular antigenic activity analogous to the effects of PPD on lymphocytes from tuberculin-sensitized patients,[20] intrinsic factor on

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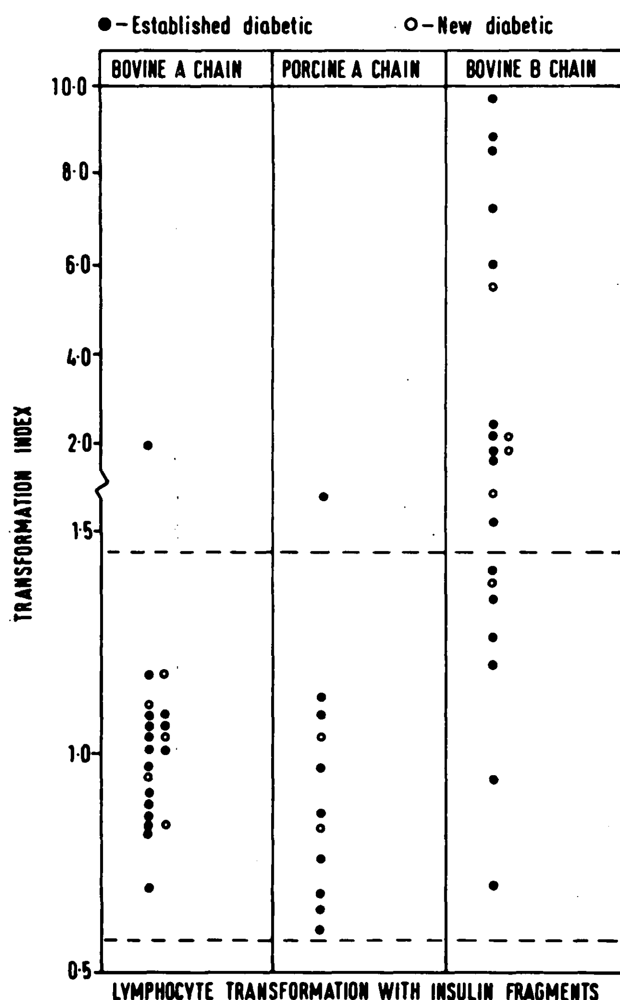

FIG. 3. Lymphocyte transformation induced by bovine and porcine A chain and bovine B chain in twenty-one diabetics who responded to intact insulin. Symbols as in figure 1. Closed circles (•)—established diabetics; open circles (o)—newly diagnosed diabetics. Significant transformation to either A chain is shown by only one diabetic, as compared to fourteen (67 per cent) who respond to B chain. The effects of B chain differ significantly ($p < 0.01$) from those of the A chain of either insulin.

lymphocytes from patients with pernicious anemia[21] and thyroglobulin on lymphocytes from patients with Hashimoto thyroiditis.[22] The blastogenic effect of insulin is perhaps not surprising in the group of established diabetics that we tested: all had been insulin-treated for months or years, virtually all[23] would be expected to have humoral antibodies to injected insulin, and it is reasonable to suppose that some have developed cellular immunity in the form of an insulin-sensitized lymphocyte subpopulation. Similar findings were reported by Halpern et al.[15] and Federlin et al.[16,17] in diabetics with insulin allergy, both of immediate (urticarial) and delayed (cutaneous) type; our own experience suggests that the phenomenon is not uncommon in diabetics who have no clinical signs of allergy. In these patients the in vitro test of lymphocyte function may be the most sensitive measurement of an insulin hypersensitivity which is not of clinical significance.

More interesting is the finding of insulin-induced lymphocyte transformation in six of ten newly diagnosed patients, four of whom had never been given insulin and two of whom had been insulin-treated for less than three weeks. Further studies are needed to confirm these observations in larger numbers of untreated diabetics but the present results at least suggest that a proportion of such patients have an insulin-sensitized lymphocyte population before exogenous insulin has been given and before[23] humoral insulin antibodies have developed. Nondiabetics do not show this cell population and its existence may reflect a state of cell-mediated autoimmunity, to insulin or insulin precursor, in early diabetes.

The virtually identical findings with bovine and porcine insulin prompted us to examine their component chains for antigenicity, and the results show a clear-cut difference between the effects of the two major chains. On the one hand, neither bovine nor porcine A chain induced significant lymphocyte transformation, and as the structure of human insulin A chain is identical to that of porcine insulin[24] it may be presumed that human A chain is also without blastogenic effect. On the other hand, bovine insulin B chain (which is identical to that of porcine insulin and differs[24] from the amino-acid sequence of human B chain only at position 30) had a striking effect in inducing blastogenesis of lymphocytes from two thirds of those patients who responded to intact insulin. The results thus suggest that B chain is the major antigenic site producing cellular hypersensitivity to insulin. Support for this hypothesis is given by the in vivo animal experiments of Clark and Munoz, who injected guinea pigs with bovine insulin, A and B chain in Freund's adjuvant: Cutaneous hypersensitivity was readily elicited by intact insulin and B chain, but A chain had no significant effect.[25]

The role of insulin B chain in cell-mediated immunity to insulin may be contrasted with the humoral response to insulin, where various studies[26,27] have suggested that the A chain is the major determinant of antibody production. It seems that differing antigenic activity, whether cellular or humoral, may reside at differing sites on the insulin molecule, such activity being perhaps governed partly by genetically determined configuration of antibody binding sites[28] and partly by the relationship between the structure of

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antibody and the amino-acid sequence against which it is directed. Finally, the apparent lack of antigenicity of A chain in our experiments might be explained by the physical changes undergone by this peptide during the splitting of insulin: Isolated A chain is 'stretched' by comparison with its configuration in the intact insulin molecule,[29] and the intra-chain disulfide bridge between six and eleven is broken with the formation of cysteic acid residues[26] which could prevent antigen-antibody reaction at this site.

Further studies should attempt to delineate more clearly the cellular antigenic portion of insulin and to indicate whether related proteins or precursors (e.g. proinsulin, C-peptide) have similar properties in early diabetes.

ACKNOWLEDGMENT

We thank the Lilly Research Laboratories for supplies of the insulins and insulin chains used for these studies.

This work is supported by a grant from the Scottish Home and Health Department.

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