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Immune response potential to poly(Tyr,Glu)-poly(DLAla)--poly(Lys) of human T cells of different donors

(T cell-replacing factor/T cell proliferation/regulation of immune response)

DVORA KATZ*, ZVI BENTWICH†, NEOMI ESHHAR†, ILANA LÖWY*‡, AND EDNA MOZES*

*Department of Chemical Immunology, The Weizmann Institute of Science; and †Department of Medicine C and Clinical Immunology, Kaplan Hospital, Rehovot, Israel

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ABSTRACT **Human peripheral blood T cells of normal donors were activated *in vitro* with autologous adherent cells pulsed with poly(LTyr,LGlu)-poly(DLAla)--poly(LLys) [abbreviated (T,G)-A--L]. The "educated" T cells were tested: (*i*) for their ability to produce a (T,G)-A--L-specific T cell-replacing factor in the cooperation with B cells for antibody responses *in vivo* or *in vitro* and (*ii*) for their ability to proliferate in the presence of a second stimulus of (T,G)-A--L. Results of screening of 66 donors demonstrated that educated T cells of about 50% of the donors produced an active (T,G)-A--L-specific factor, whereas activated cells of only half of the factor producers were capable of proliferating in the presence of the antigen. Thus, as reported for all other species studied, human individuals differ in their response potential to (T,G)-A--L.**

Immune response (*Ir*) genes have been found in a variety of species (1–4) and therefore it is widely assumed that they must exist in humans. For the majority of antigenic systems studied, *Ir* genes were found to be linked to the major histocompatibility complex (MHC) of the species (1, 2, 5–8). Hence, it is likely that *Ir* genes in the human will map close to the *HLA-D* locus because of its similarity to the *I* region of the mouse (9). The *Ir* genes also appear to be the obvious candidates to explain involvement of the MHC in disease (10). In spite of the paramount importance of understanding the function of putative *Ir* genes in humans, the existence of such genes has not been established yet, probably due to the complexity of the antigenic systems that play a role in disease occurrence (10, 11) and due to the ethical constraints on immunizing humans with antigens.

One of the antigenic systems most extensively used for studies on the genetic regulation of immune responsiveness in a variety of species is that of the synthetic polypeptide antigen poly(LTyr,LGlu)-poly(DLAla)--poly(Lys), abbreviated as (T,G)-A--L (1, 2, 5, 12, 13). In the mouse, antibody responses (2, 5), delayed-type hypersensitivity (DTH) reactions (14), and antigen-dependent proliferation responses (15) specific to (T,G)-A--L were all found to be genetically controlled. In addition, an antigen-specific T cell-replacing factor that could cooperate with B cells for the production of (T,G)-A--L-specific antibodies has been reported for this synthetic polypeptide (16, 17). One of the properties of antigen-specific T cell-replacing factors is their ability to interact with B cells across major histocompatibility (H-2) "barriers" (16, 17), although their production is regulated by *Ir* genes (16, 18) and they possess Ia determinants (17, 19). Furthermore, it has been shown that these factors are not species specific (20, 21). It appeared, therefore, that the (T,G)-A--L antigenic system is most adequate for attempts to study genetically controlled immune responses in humans.

In the present study we have generated *in vitro* "educated" human peripheral blood leukocytes (PBL) of normal donors to (T,G)-A--L. The educated cells were tested for their ability to produce a helper factor. Because the factors are not species specific (20, 21), it was possible to measure their activity by their ability to cooperate with murine B cells for antibody production *in vivo* or *in vitro*. Furthermore, the (T,G)-A--L-activated PBL were also tested for their proliferation potential in response to a second stimulus of (T,G)-A--L *in vitro*. We report here that educated cells of about 50% of the donors produced (T,G)-A--L-specific factors that could replace helper T cells in the process of antibody production. The PBL of about half of the individuals whose cells produced these factors also proliferated specifically in the presence of (T,G)-A--L. Thus, the difference between the response potential of individuals to (T,G)-A--L suggests that the response to this antigen in man might also be under *Ir*-gene control.

MATERIALS AND METHODS

Mice. The inbred mouse strains C3H.SW and CWB (H-2^b) were obtained from the experimental animal unit, the Weizmann Institute of Science, Rehovot, Israel.

Antigens. The synthesis and characterization of the antigen (T,G)-A--L have been described (22). 4-Hydroxy-5-iodo-3-nitrophenylacetyl-ovalbumin (NIP-OVA), NIP-human gamma globulin (NIP-HGG), and NIP-(T,G)-A--L were used as well.

***In Vitro* Education of Human PBL.** Human PBL were obtained from healthy donors by separation of the blood over Ficoll/Hypaque gradients. The cells were educated *in vitro* according to the procedure described for murine cells (23). The PBL were suspended in RPMI-1640 medium (Bio-Lab, Israel) supplemented with 10% fetal calf serum (GIBCO), 2 mM glutamine, penicillin at 100 units/ml, streptomycin at 100 μg/ml, 1 mM Hepes at pH 7.3, nonessential amino acids, and 50 μM 2-mercaptoethanol (RPMI/fetal calf serum medium). Adherent cells were prepared by incubating 60×10^6 PBL suspended in 3 ml of minimal Eagle's medium (Bio-Lab) supplemented with 5% fetal calf serum in 60-mm Petri dishes (Nunc, Roskilde, Denmark) for 90 min at 37°C. Nonadherent cells were removed and the washed adherent cells were incubated with 4 ml of (T,G)-A--L at 4 μg/ml. After 1-hr incubation at 37°C, unbound antigen was removed and the plates were washed three times with minimal Eagle's medium. Four milliliters of the nonadherent cells at a concentration of 10^7 cells per ml in RPMI/fetal calf serum medium were seeded on the antigen-bearing adherent cells. The cells were incubated at 37°C in a

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Abbreviations: (T,G)-A--L, poly(LTyr,LGlu)-poly(DLAla)--poly(LLys); *Ir* genes, immune response genes; MHC, major histocompatibility complex; NIP, 4-hydroxy-5-iodo-3-nitrophenylacetyl; OVA, ovalbumin; HGG, human gamma globulin; PFC, plaque-forming cells; PBL, peripheral blood leukocytes; SRBC, sheep erythrocytes.

‡ Present address: Institut Pasteur, Paris, France.

humidified incubator with a constant gas flow of 5% CO_2 for 5 days. Cultures were fed with RPMI/fetal calf serum medium every second day.

Preparation of Helper Factor. Educated cells were centrifuged after 5 days in culture and resuspended in minimal Eagle's medium at 5-10 × 10^6 cells per ml and incubated for 12–18 hr at 37°C in the presence of (T,G)-A--L at 2 μg/ml. After this incubation period the cells were removed by centrifugation and their supernatants were tested for T cell helper activity.

***In Vivo* Antibody Production.** The presence of functional helper factors was tested by injecting a mixture of factors, anti-Thy-1.2 and complement-treated spleen cells primed to (T,G)-A--L, and 10 μg of antigen into the tail vein of irradiated (800 roentgens; 1 roentegen = 2.6 × 10^{-4} coulomb/kg) mice syngeneic for the B cell donors. Eight to 14 days after the cell transfer, the recipient animals were bled and their sera were tested for specific antibodies.

***In Vitro* Antibody Production Assay.** A million NIP-OVA-primed spleen cells and either 0.25 × 10^6 (T,G)-A--L-primed spleen cells or 50 μl of supernatants of activated cells were put into the wells of microtiter plates (Falcon) together with 0.1 μg of NIP-(T,G)-A--L. Culture conditions were according to Mishell and Dutton (24). The number of plaque-forming cells (PFC) in the cultures was determined in Cunningham chambers (25), using NIP-derivatized sheep erythrocytes (SRBC) as target cells. Numbers of PFC are expressed as the mean ± SD of plaques per 10^6 viable cells.

Antigen-Specific Proliferative Response of (T,G)-A--L-Educated PBL. The assay was carried out in flat-bottom microtiter plates (Falcon), using 2 × 10^5 educated cells per well in the presence of 2% autologous adherent cells and various concentrations of antigen in a final volume of 200 μl. Phytohemagglutinin M (Difco) was used as control for culture conditions in each experiment. Triplicate cultures were incubated in 5% CO_2 at 37°C for 7 days. Eighteen hours before harvesting the cells, [^{3}H]thymidine was added at 20 μCi per well (1 Ci = 3.7 × 10^{10} becquerels). Incorporation of [^{3}H]thymidine was determined in a Packard scintillation counter.

Measurement of Anti-(T,G)-A--L Antibodies by Solid-Phase Radioimmunoassay. The activity of anti-(T,G)-A--L antibodies in the sera of mice was determined by a modification of a solid-phase binding assay (23). Flexible plastic microtiter plates were coated with (T,G)-A--L (100 μl of a solution of 50 μg/ml). After 1- to 2-hr incubation at room temperature, plates were washed twice with phosphate-buffered saline (0.01 M sodium phosphate buffer/0.15 M NaCl, pH 7.2) containing 5% fetal calf serum. Twenty-five microliters of various dilutions of antisera were then added for an additional 2 hr of incubation. Plates were washed thrice and incubated with 25 μl of ^{125}I-labeled purified goat anti-mouse Fab antibodies at 4°C for 18 hr. After extensive wash, plates were dried and wells were cut out of the plates and their radioactivities were measured in a gamma counter (Packard).

Antigen-Sepharose Columns. (T,G)-A--L was coupled to activated Sepharose (26) at a concentration of 10 mg of antigen per g of Sepharose with a yield of coupling of 95%. The antigen-specific T cell helper supernatants were passed through the (T,G)-A--L Sepharose column. The effluents were collected, and then the columns were washed with phosphate-buffered saline and eluted with 0.1 M NH_4OH.

RESULTS

***In Vivo* Helper Activity of a (T,G)-A--L-Specific Factor.** PBL were collected from a number of normal donors and activated *in vitro* for 5 days in the presence of (T,G)-A--L as described above. Supernatants of the antigen-activated cells were collected after an additional incubation in the presence of (T,G)-A--L for 12–18 hr and tested for their capability to replace (T,G)-A--L-specific helper T cells in the cooperation with antigen-primed B cells in the process of antibody production in adoptive transfer experiments. A number of such experiments are represented in Table 1. As can be seen in the table, four of the representative supernatants were capable of cooperating with (T,G)-A--L-primed B cells; three nonfunctional supernatants are shown for comparison. About 40% of the supernatants tested were found to possess (T,G)-A--L-specific helper activity. The same donors were tested two to five times for the capability of their (T,G)-A--L-educated cells to produce an active helper factor, and the results were fully reproducible.

The results shown in Table 1 suggested that it is possible to activate human peripheral T cells towards a specific antigen and that educated cells of some of the donors produce an antigen-specific factor. However, the amount of factor required for a transfer experiment is large because the factor injected into one animal was an equivalent of 5–10 × 10^6 viable cells and at least five mice were injected in each group. In addition, the helper activity of the factor *in vivo* was usually lower than that of helper T cells. Therefore, we have determined the activity of the helper factor in an *in vitro* antibody production system as well.

***In Vitro* Helper Activity of (T,G)-A--L-Specific Human T Cell Factors.** The supernatants of (T,G)-A--L-activated peripheral blood leukocytes of different donors were tested for their T cell helper activity in a carrier-hapten *in vitro* antibody production system. Thus, the supernatants were checked for their ability to cooperate with NIP-OVA-primed spleen cells (used

Table 1. Helper activity of supernatants of (T,G)-A--L-educated human PBL in the production of specific antibodies *in vivo*

Cells transferred into irradiated recipients*	Production of anti-(T,G)-A--L antibodies, cpm†						
	A.F.‡	T.W.	A.B.N.	E.L.‡	S.S.‡	K.I.	B.M.G.
20 × 10^6 spleen cells of high-responder mice primed to (T,G)-A--L	7164 ± 377	5175 ± 286	14,499 ± 402	12,401 ± 1,086	12,282 ± 357	5434 ± 2817	13,892 ± 3,606
20 × 10^6 cells of high-responder mice primed to (T,G)-A--L§	686 ± 76	1183 ± 101	449 ± 201	8,257 ± 861	5,190 ± 233	2299 ± 750	1,776 ± 1,159
20 × 10^6 B cells + factor¶	2884 ± 561	5874 ± 161	3,202 ± 1,334	13,114 ± 666	3,703 ± 1,181	2092 ± 517	1,683 ± 580

* Cells were transferred intravenously into irradiated (800 rads) high-responder mice of the *H-2*^{b} haplotype together with 100 μg of (T,G)-A--L. The recipients were bled 8–14 days after transfer and antisera of individual mice were checked for (T,G)-A--L-specific antibody activity.

† Antibody production was measured by solid-phase radioimmunoassay. Results are expressed as cpm of ^{125}I-labeled goat anti-mouse Fab bound (mean of triplicate wells). The results represent the mean (±SD) response of four to six mice in a group.

‡ The factors of A.F., E.L., and S.S. were also tested for *in vitro* helper activity, with full concordance of the results.

§ High-responder primed spleen cells treated with anti-Thy-1.2 + complement.

¶ One milliliter of supernatant of 5–10 × 10^6 *in vitro* (T,G)-A--L-educated human PBL.

Table 2. Helper activity of (T,G)-A--L-specific supernatant of human educated PBL in the *in vitro* antibody production assay

NIP-OVA-primed spleen cells cultured with*	NIP-specific PFC per 10^6 viable cells†							
	I.R.	E.L.‡	A.B.I.	A.F.‡	Av.Fr.	R.Z.	S.S.‡	A.L.
—	85 ± 25	138 ± 73	26 ± 0	95 ± 47	56 ± 5	104 ± 39	34 ± 0	68 ± 50
NIP-OVA	600 ± 122	526 ± 37	1866 ± 20	1439 ± 303	2122 ± 61	1142 ± 181	695 ± 96	1794 ± 276
NIP-HGG	184 ± 33	269 ± 18	305 ± 27	202 ± 25	72 ± 8	176 ± 71	53 ± 0	175 ± 47
NIP-(T,G)-A--L	156 ± 61	129 ± 66	368 ± 105	184 ± 87	104 ± 10	202 ± 78	58 ± 0	279 ± 86
(T,G)-A--L-primed spleen cells + NIP-HGG	184 ± 56	258 ± 11	226 ± 41	250 ± 105	54 ± 16	196 ± 58	62 ± 23	315 ± 36
(T,G)-A--L-primed spleen cells + NIP-(T,G)-A--L	475 ± 148	623 ± 11	809 ± 95	970 ± 214	711 ± 61	733 ± 105	390 ± 137	1333 ± 151
Factor§ + NIP-HGG	–	171 ± 37	—	41 ± 0	272 ± 51	69 ± 33	56 ± 19	134 ± 70
Factor§ + NIP-(T,G)-A--L	602 ± 137	566 ± 73	1250 ± 62	500 ± 159	742 ± 86	637 ± 112	47 ± 16	102 ± 58

* NIP-OVA-primed spleen cells were cultured in microtiter plates together with either (T,G)-A--L-primed spleen cells or the factor. One microgram of NIP-OVA, NIP-HGG, or NIP-(T,G)-A--L was added to each well.

† Indirect PFC were determined by using NIP-SRBC. Results are expressed as the mean (±SD) of quadruplicate tests. Underlining indicates high responses in all tables.

‡ Tested also for *in vivo* helper activity.

§ Fifty microliters of factor was added to each well. One milliliter of factor is the equivalent of 10^7 human (T,G)-A--L-educated PBL.

as a source of B cells) for eliciting NIP-specific antibodies when NIP-(T,G)-A--L was given in the cultures. Table 2 demonstrates six representative supernatants that could replace (T,G)-A--L-specific T cells for production of antibodies *in vitro*. For comparison two experiments with nonfunctional supernatants (S.S and A.L.) are shown in the table as well. The activity of the functional supernatants was comparable to that of (T,G)-A--L-primed T cells, and it was antigen specific because the supernatants did not cooperate with NIP-OVA spleen cells when the antigen added to the culture was NIP-HGG (Table 2). Cells of each donor were tested two to six times for their ability to produce the (T,G)-A--L-specific helper factor after the *in vitro* education in the presence of the antigen, and the results were always reproducible. As indicated in Tables 1 and 2, supernatants of three of the donors were tested for their helper activity both *in vivo* and *in vitro*, and the results of the two assays agreed. Table 3 demonstrates the reproducible helper activity of factors obtained from cells of the same donor after distinct education experiments on different dates. As can be seen in the table, the specific helper activity is evident in all the factor preparations. An additional reproducible experiment with factor obtained from the same donor (E.H.) is shown in Table 4.

In a very small number of experiments the factor had a low nonspecific helper activity as in the case of donor Av.Fr. (Table 2). However, in all these instances, as in the case of Av.Fr., the specific activity of the factor was significantly higher. Moreover, the specific helper activity was always reproducible, whereas the low nonspecific helper activity could not be demonstrated in the different batches of the factors obtained from the same donor after activation to (T,G)-A--L.

To date, supernatants of activated cells of 66 donors have been tested *in vitro* for their ability to replace T cells in antibody production, and the cells from 50% of the donors have been found to produce an active antigen-specific factor.

We thus conclude that normal human donors can be screened according to the potential of their T cells to be educated to (T,G)-A--L and to produce helper T cells factors.

Antigen Specificity of the Human T Cell Helper Factors. In order to confirm the antigen specificity of the human T cell factor, active supernatants of a few donors were passed through columns of Sepharose to which (T,G)-A--L was coupled. Table 4 demonstrates that the activity of the supernatants was removed by (T,G)-A--L-Sepharose as indicated by the lack of

Table 3. Reproducibility of the helper activity of different preparations of (T,G)-A--L-specific factor from the same donor (E.H.)

NIP-OVA-primed spleen cells cultured with	NIP-specific PFC per 10^6 viable cells*		
	Exp. 1	Exp. 2	Exp. 3
—	129 ± 45	192 ± 40	236 ± 82
NIP-OVA	1857 ± 171	542 ± 51	1166 ± 277
NIP-HGG	320 ± 80	159 ± 63	128 ± 76
NIP-(T,G)-A--L	318 ± 101	114 ± 57	177 ± 79
(T,G)-A--L-primed spleen cells + NIP-HGG	333 ± 78	333 ± 60	125 ± 61
(T,G)-A--L-primed spleen cells + NIP-(T,G)-A--L	1119 ± 182	970 ± 158	888 ± 99
Factor + NIP-HGG	183 ± 98	115 ± 56	203 ± 48
Factor + NIP-(T,G)-A--L	1130 ± 37	526 ± 38	1461 ± 172

* Indirect PFC were determined by using NIP-SRBC indicator cells. Results are expressed as the mean (±SD) of quadruplicate tests. Each experiment was performed with a new preparation of factor.

Table 4. Human helper T cell factors are (T,G)-A--L specific

NIP-OVA-primed spleen cells cultured with	NIP-specific PFC per 10^6 viable cells*	
	E.H.	V.F.
—	120 ± 14	113 ± 44
NIP-OVA	1256 ± 58	1069 ± 125
NIP-HGG	133 ± 23	117 ± 33
NIP-(T,G)-A--L	125 ± 16	116 ± 12
(T,G)-A--L-primed spleen cells + NIP-HGG	156 ± 10	231 ± 35
(T,G)-A--L-primed spleen cells + NIP-(T,G)-A--L	615 ± 61	687 ± 134
Factor + NIP-HGG	160 ± 63	311 ± 25
Factor + NIP-(T,G)-A--L	764 ± 92	820 ± 147
Effluent from (T,G)-A--L-Sepharose + NIP-(T,G)-A--L	243 ± 30	333 ± 56
Eluate from (T,G)-A--L-Sepharose† + NIP-(T,G)-A--L	434 ± 39	862 ± 126

* Indirect PFC were determined by using NIP-SRBC indicator cells. Results are expressed as the mean (±SD) of quadruplicate tests.

† Elution was performed with 0.1 M NH_4OH.

Table 5. Antigen-specific proliferative response of human PBL educated to (T,G)-A--L

Cell donors	[^{3}H]Thymidine incorporation at the indicated concentration of (T,G)-A--L, cpm					
	0 μg/well	3.125 μg/well	6.25 μg/well	12.5 μg/well	25 μg/well	50 μg/well
N.I.	191 ± 86	—	3,538 ± 790	3192 ± 624	3113 ± 350	2732 ± 330
A.B.I.	1696 ± 213	—	13,022 ± 988	4045 ± 1965	3785 ± 442	3211 ± 837
V.F.	1528 ± 45	4635 ± 544	4,160 ± 240	2532 ± 210	—	2338 ± 935
T.W.	982 ± 175	—	793 ± 81	986 ± 65	1000 ± 182	837 ± 173
B.M.G.	1604 ± 332	1241 ± 169	1,262 ± 16	1089 ± 227	1149 ± 258	—

* Results are expressed as mean (±SD) of triplicate tests.

helper activity in the effluents of the column. However, the factors eluted from the column with 0.1 M NH_4OH possessed the helper activity of the original supernatants. Thus, the (T,G)-A--L specificity of the helper factors has been confirmed.

Antigen-Dependent Proliferation of the (T,G)-A--L-Activated Human PBL. The (T,G)-A--L *in vitro* activated human PBL were also tested for their ability to proliferate after a second stimulus with the (T,G)-A--L. Out of 34 activated cell cultures tested for proliferation only 8 responded to (T,G)-A--L. The stimulation indices observed ranged between 3 and 15. Uneducated cells of the above donors were also tested, and none of them responded by proliferation in the presence of (T,G)-A--L. All activated cell cultures were tested for their proliferation potential to the T cell mitogen phytohemagglutinin as control for culture conditions, and all of them [responders and nonresponders to (T,G)-A--L] responded well to the mitogen, with stimulation indices ranging between 8 and 40. Table 5 demonstrates representative results obtained with activated cell cultures of five out of the donors tested. The three first representative cultures produced also active helper factors. Activated cells of the fourth donor produced an active (T,G)-A--L-specific helper factor (Table 1) but did not respond in proliferation (Table 5), and the cells of the last donor neither produced a helper factor nor proliferated in the presence of (T,G)-A--L. It is noteworthy that of the limited number of donors tested, we did not detect a case in which activated cells were capable of (T,G)-A--L-specific proliferation but did not produce a helper factor.

DISCUSSION

The main finding of the present report is that human individuals differ in their immune response potential to the synthetic antigen (T,G)-A--L, suggesting that, as demonstrated for all other species studied, the ability of humans to respond to (T,G)-A--L is genetically regulated.

The establishment of a system in which human PBL are activated *in vitro* to the synthetic antigen (T,G)-A--L enabled the study of the immune response potential to this antigen in a large number of individuals. The activated cells are most likely T cells, because their products replace T cell function and, when characterized, more than 95% of them formed erythrocyte-rosettes and did not react with antisera against human immunoglobulins.

The (T,G)-A--L-educated cells of responders were found to produce an antigen-specific T cell-replacing factor that could be tested for its activity by its potential to specifically trigger murine high-responder B cells. The fact that the T cell factor was found to be xenoreactive facilitated the examination of the immune response potential of human T cells: murine B cells, which are known to respond to a (T,G)-A--L T cell signal (16), were used to check the activity of the factor rather than human B cells, whose immune response to (T,G)-A--L is unknown. The observation that antigen-specific T cell-replacing factors may cooperate with B cells across species barrier had been made previously. It has been reported that T cell factors of murine origin can be absorbed on human PBL of certain donors (27) and, vice versa, human T cell factors were shown to activate murine B cells for antibody production (21, 28). Heijnen and coworkers (29, 30) have demonstrated collaboration between human helper T cell factors and human B cells; however, they worked with antigenic systems to which the response is not genetically controlled and all individuals were expected to respond to them.

In the present study we have demonstrated that about 50% of the donors possessed T cells that could be activated to (T,G)-A--L to produce a functional helper factor (Tables 1 and 2). T cells of only 50% of these donors could proliferate in response to a second stimulus of (T,G)-A--L *in vitro* (Table 5). These observations are in agreement with results of studies on the immune response of mice to (T,G)-A--L. Only mice possessing the $H\text{-}2^b$ haplotype are high responders to (T,G)-A--L as manifested in antibody production (5), in delayed type hypersensitivity responses (14), and in the ability of their sensitized T cells to proliferate in the presence of a second stimulus of antigen (15). However, mice with the $H\text{-}2^k$ haplotype are low responders for all the above mentioned functions (5, 14, 15) but their T cells can be educated to (T,G)-A--L to produce a helper factor (16), in analogy to those individuals whose T cells produce an active factor but do not proliferate. And, finally, strains of mice exist (i.e., $H\text{-}2^s$) that do not respond to (T,G)-A--L by any of the above-mentioned functions (5, 14, 15), including factor production (18), as we have demonstrated for about 50% of the donors tested.

It should be noted that none of the T cells tested proliferated in the presence of (T,G)-A--L without being previously sensitized *in vitro*. These results are in contrast to the report by Young and Engleman (31), who demonstrated proliferation responses to (T,G)-A--L and to poly(LGlu^{60}LAla^{30}LTyr^{10}) of T cells of individuals who were not previously deliberately immunized to the antigens.

The fact that individual donors differ in their ability to react to (T,G)-A--L enables a linkage study between the immune response potential to this antigen and the major histocompatibility (HLA) complex of man: HLA types of responder and nonresponder donors of a random population as well as of family members can be determined on the basis of these results.

The antigen-specific T cell helper factor is antigen specific, as shown by the fact that it cooperates with B cells to produce NIP-specific antibodies only when the carrier is (T,G)-A--L and not when NIP-HGG is used as the boosting antigen in culture (Table 2). Furthermore, the activity of the factor is removed by a (T,G)-A--L-Sepharose column and is fully recovered in the eluate of this column (Table 4). The availability of such T cell products permits molecular characterization of the human T cell recognition system.

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