#147

T Cell Hybrids with T Cell Functions

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A. Introduction

Different T cell functions, e.g. help, suppression and cytotoxicity are associated with different T cell subsets, bearing distinctive differentiation markers. Thus effector T cells mediating help are Ly $1^+2^-3^-$ and may bear Ia determinants, those mediating suppression are Ly $1^-2^+3^+$, IJ^+, and cytotoxic cells are Ly $1^-2^+3^+$ and do not bear Ia determinants (1). At least the cytotoxic effectors are Ly 6.2 positive, but they arise from Ly 6.2 negative precursors (2). Thus expression of certain differentiation markers (Ly 123) is associated with acquisition of the ability to perform a particular function, even before encounter with antigen, whilst others are expressed as a result of further differentiation, including clonal expansion, following response to antigen (e.g. Ly 6.2 and possibly Ia antigens).

The successful hybridization of primed B cells with a B cell tumour to produce functional antibody secreting hybrids appears to require that each of the two populations be at the same stage of differentiation (3). It is possible that the same restrictions may apply to production of functional T cell hybrids between normal primed T cell populations and a T cell tumour. There may be additional restrictions on the formation of hybrids between T cells capable of carrying out certain functions, such as cytotoxicity, since it could be that the very act of fusion between a cytotoxic cell and a tumour cell would lead to the killing of the tumour cell (4) and thus prevent hybrid formation. However, there is no reason to assume that T cells with other functions e.g. help and suppression cannot be successfully fused with an appropriate T cell tumour, and function be expressed.

We have used the AKR thymoma BW5147 to make somatic cell hybrids with T cell populations induced *in vitro* to express (a) cytotoxicity, (b) antigen specific suppression and (c) antigen specific help. In each case, we have obtained hybrids which carry the Thy 1 marker of each parental type and for suppression and help we have obtained T cell hybrids which express the function shown by the original primed normal parental T cell population. Typing of other cell surface markers has yielded some rather surprising and unexpected results: some hybrids express apparently 'inappropriate' differentiation antigens and fail to express differentiation antigens which would be appropriate to the function their supernatants show.

Finding of immunologically functional material in the supernatants of hybrids growing *in vitro* has allowed us to do preliminary analyses of some immunochemical characteristics of the functional moieties. These and further investigations should throw substantial light on the question of the nature of the T cell receptor(s).

B. MATERIALS AND METHODS

I. Animals and Antigens

Mice. CBA/Ca, C57BL/10, B10.BR, AKR, A.TH, A.TL, SJL, $(B6xBALB/c)F_1$, B6.Ly1.1, and

(A.Thyl.1x CBA) F_1 mice were obtained from the Division of Comparative Medicine, CRC and from the Imperial Cancer Research Fund Breeding Unit. The antigens used were keyhole limpet haemocyanin (KLH, a gift from Dr M. Rittenberg, Portland, Oregon), a copolymer of L-glutamic acid ^{60}-L-alanine ^{30}-L-tyrosine ^{10}(GAT, a gift from Dr P. Maurer, Philadelphia) and poly(L-tyrosine-L-glutamine)-poly(D-L alanine) -poly(L-lysine) [(T,G(-A--L) a gift from Dr Edna Moses, the Weizmann Institute, Rehovot]. Trinitrophenylated KLH and GAT had 8 and 4 groups of TNP per 100,000 daltons. Trinitrophenylated polyacrylamide beads (TNP-PAA), a thymus independent antigen, were prepared by Dr Marilyn Baltz at University College London. The preparation of TNP antigens was as described earlier (5).

II. T Cell Tumour Line

In every case the tumour line used for the fusions has been BW5147 (BW). This was obtained from Dr Robert Hyman, La Jolla via Prof. L.A. Herzenberg of Stanford in September 1976. It was HGPRT negative, thus allowing for suppression of its growth in HAT medium (see below) following fusion with a source of normal T cells: non-fused T cells die out in a matter of days, and only hybrids between these cells and BW grow in HAT. BW and all fusion hybrids were maintained in Dulbecco's Modification of Eagle's Medium DMEM, Flow Cat. No. 12-332-54(1-001M) with 10% foetal calf serum (FCS) obtained from Gibco (Scotland) and screened for its ability to support mixed lymphocyte responses for the generation of cytotoxic cells, i.e. our FCS was not specially screened for fusion work. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere.

III. Normal Primed T Cell Populations

Spleen cells were used as a source of normal T cells: they were primed *in vitro* in the following ways:- (1) for cytotoxicity, either 25×10^6 AKR or CBA spleen cells were co-cultured in tissue culture flasks in a humidified 10% CO_2 atmosphere at 37°C for 4 or 5 days with equal numbers of 2000R irradiated C57BL/10 spleen cells in bicarbonate buffered RPMI medium containing 10% FCS, 5×10^{-5}M 2ME, 10mM of Hepes, glutamine, penicillin and streptomycin; (2) for suppression, CBA spleen cells were cultured in the inserts of Marbrook Diener flasks for 4 days with 100 µg of KLH/ml in Eagles Minimum Essential Medium (MEM, Gibco, Cat. No. F15), containing 3% FCS and Hepes at 4.36g/litre. The same medium, but with bicarbonate added at 3.7g/litre instead of Hepes, was used in the outer chamber (6); and (3) for help, B10 spleen cells were cultured as above in Marbrook Diener flasks, but with (T,G)-A--L at 1 µg/ml (7).

IV. Cell Hybridization

The cell hybridizations, as previously described (3,8) were performed with polyethylene glycol. (PEG, BDH MW 1500, 50% in BSS, pH 7.8: to prepare, 25 gm PEG crystals were autoclaved, and 25 mls of warmed balanced salt solution (BSS) with no foetal calf serum were added at 56°. The solution can be used immediately or can be stored at +4°C for several months). 10^8 *in vitro* primed cells and 10^7 BW5147 cells were washed twice in serum free BSS and pelleted together at 400 g. 0.5ml of PEG was added slowly (0.1ml per 10 seconds) over a period of 1-2 minutes as the cells were gently shaken into suspension, and 0.5ml of serum free BSS was then added at the same rate. A further 5ml of BSS were then added dropwise before slowly filling the tube to 20ml with BSS. The cells were spun at 400g, the supernatant discarded and the cells resuspended in 100ml of DMEM with 20% FCS. The cell suspension was dispensed in 2ml aliquots into 48 wells of two 24 well Linbro trays (Cat. No. Flow FM 1624TC) and incubated at 37°C in a humidified atmosphere of 5% CO_2. 24 hours after the fusion 1.0ml of medium was removed from each well and replaced by 1.0ml HAT (DMEM plus 20% FCS, 1×10^{-4}M hypoxanthine, 1.6×10^{-5}M thymidine and 4×10^{-7}M aminopterin) and this procedure was repeated 48 and 72 hours after fusion. On the sixth, eighth and tenth day the medium was

changed to HT (DMEM plus 20% FCS, $1x10^{-4}$M hypoxanthine and $1.6x10^{-5}$M thymidine). Thereafter (day 13 from fusion) the medium was changed to DMEM plus 10% FCS, and the contents of each Linbro well which started to grow within the next 1-2 weeks were subcultured in Linbro wells and then transferred to Nunc tissue culture flasks (50ml, Nunclon-Delta 1461). Aliquots of supernatants were tested for function when the cells were growing in the Linbro plates, and subsequently in the flasks, in the case of the suppressor and helper hybrids, whilst in the case of hybrids made with cytotoxic populations, functional testing for cytotoxicity using ^{51}Cr labelled target cells was done with hybrid cells as attackers following growth and transfer of hybrid lines to flasks.

V. Cell Cultures for Functional Assay of Hybridoma Products

Only supernatants of hybridoma cultures were initially tested for suppression and help. For the assay Marbrook Diener cultures incubated under the conditions described above were used. The assay for suppression was to add the hybridoma supernatants, at a 10% or 1% final concentration, to the mixture of KLH, GAT or (T,G)-A--L primed helper cells (^{HC}KLH, ^{HC}GAT, or ^{HC}(T,G)-A--L) primed *in vitro* as described previously (6,7,9), normal spleen cells, and haptenated antigens TNP-KLH, DNP-GAT or DNP-(T,G)-A--L. The anti-DNP antibody forming cells (AFC) were assayed on day 4 of the *in vitro* cooperative culture (7). Using unprimed B cells only IgM anti-DNP AFC were detected. All cultures were in triplicate. The assay is summarised as: spleen cells + haptenated antigen = background, helper cells + spleen cells + haptenated antigen = help, helper cells + spleen cells + haptenated antigen + suppressor cell S/N = suppression.

The results are expressed as numbers of anti-DNP AFC/culture ± SE. The degree of suppression was counted as

$$\% \text{ suppression} = 100 - \frac{(\text{suppression} - \text{background})}{(\text{help} - \text{background})} \times 100$$

The assay for helper cell hybridoma products was to add instead of the helper cells the hybridoma supernatants at 1% or 0.1% final concentration to a mixture of normal spleen cells and antigen, 1 μg/ml DNP-(T,G)-A--L. Anti-DNP AFC were assayed on day4.

VI. Cell Surface Antigens

The following antisera were used to test for cell surface antigens Thy 1.1, Thy 1.2, Ly 1.1, Ly 1.2, Ly 2.1, Ly 6.2, H-2^k and Ia^k on parental and hybridoma cells: Thy 1 antisera were prepared conventionally by injecting CBA thymocytes into AKR mice (Thy 1.2) and AKR thymocytes into CBA mice (Thy 1.1) and obtaining hyperimmune serum or ascites fluid after 6 or more immunizations. Ly 1.1 antisera were obtained by hyperimmunizing (B6xBALB/c)F_1 mice with thymocytes from B6 Ly 1.1 mice. Ly 1.2 and Ly 2.1 antisera were the kind gift of Dr E.A. Boyse, Sloan Kettering Institute. Ly 6.2 antisera were obtained by hyperimmunizing (A. Thy 1.1xCBA)F_1 mice with thymocytes and spleen cells from AKR mice. Ia^k antisera were prepared by hyperimmunizing A.TH mice with A.TL spleen cells.

The possession of cell surface antigens Thy 1.1, Thy 1.2, H-2^k and Ia^k were tested by direct complement mediated cytotoxicity using ^{51}Cr labelled hybrids as target cells.In addition, a quantitative absorption technique was used for testing for the presence of Ly 1.1, Ly 1.2, Ly 2.1, Ly 6.2 and Ia^k on the cell surface of hybrids and their parental cells. Briefly, each of the antisera were first titrated by a micro-trypan blue exclusion test on appropriate targets, using thymocytes for Ly 1.1 1.2 and 2.1, spleen cells for Ia^k and lymph node cells for Ly 6.2, and a dilution of each antiserum was then chosen which still just showed plateau level killing: 50 μl aliquots of these dilutions of antisera were then absorbed for 1 hour at RT with 5 different numbers, usually $50x10^6$, $25x10^6$, $12.5x10^6$, $6.25x10^6$ and $3.12x10^6$, of hybrid cells and appropriate control cells, and the supernatants then tested on

the indicator target cells, using the micro-trypan blue exclusion test.

VII. Immunoabsorptions

One of the suppressor cell hybridoma products, S1.41 supernatant, SFS1.41, was subjected to detailed functional and immunochemical analyses. SFS1.41 was taken from bulk cultures of this hybrid 9 months after the fusion. It was absorbed and 'acid eluted', by using Sorensen's buffer pH 2.4, from the following immunoabsorbents: KLH (1mg of KLH/1ml beads), GAT (1mg of GAT/1ml beads), anti Ia^k (0.5ml of the anti Ia^k serum described above/5ml beads) anti Ia^s (ATL anti ATH serum kindly donated by Dr McKenzie, Canberra, Australia, 0.5ml of serum/5ml beads), anti $I\text{-}J^k$ (B10.HTT anti B10.S (9R) serum donated by Dr I. McKenzie, 0.5ml of serum/5ml beads anti $I\text{-}J^s$ (B10.S(9R) anti B10.HTT serum donated by Dr I. McKenzie, 0.5ml of serum/ 5 ml beads, and anti MIg (0.5ml of rabbit anti mouse Ig/5ml beads). The beads used were Sepharose 4B (Pharmacia, Sweden). The cyanogen bromide activation of beads and coupling of antigen or antisera was as described (10).

VIII. Anti suppressor Factor Antisera

The preparation of these antisera and their characteristics have been recently described (11). The antisera made in rabbits (R α SF) against KLH specific suppressor factor of CBA origin seems to recognise a constant region like determinant in all suppressor factors, while the antibody made in CBA mice (M α SF) recognises idiotypic determinant(s) unique to the KLH specific suppressor factor of CBA mice. These antisera were used at a 0.1% final concentration.

C. RESULTS

Table 1 summarizes the results in terms of the numbers of hybrids recovered from each type of hybridization, the cell surface markers shown by some of these hybrids and the number of functional hybrids obtained.

Table 1. Summary of Results (* number positive/number tested; + by adsorption; o by cytotoxicity; NT = not tested)

Normal parent function	No of wells set up	No of wells with hybrids	Thy1.1 + Thy1.2	Ia^k	$H\text{-}2^k$	Ly1.1	Ly1.2	Ly2.1	Ly6.2	specific	non-specific	specificity unknown
Cytotoxicity $H\text{-}2^k$ anti $H\text{-}2^b$	144	60	$11/12^{*o}$	$0/20^{o}$	NT	NT	NT	NT	NT	0	0	
Suppression CBA-KLHSC	96	14	$3/13^{o}$	$0/2^{+}$	$2/2^{o}$	$1/2^{+}$	$1/2^{+}$	$0/2^{+}$	$2/2^{+}$	2	2	4
Help B10 (T,G)-A--LHC	48	19	NT	NT	NT	NT	NT	NT	NT			12

I. Cytotoxicity

We have reported earlier that the majority of hybrids tested following fusion of BW with mixed lymphocyte culture cells had the Thy 1 markers of both the tumour parent (Thy 1.1) and the normal parent (Thy 1.2) (12). This indicated to us that they were probably T cell hybrids, although this type of evidence is merely circumstantial. Ia antigens were never found on the cell surface of these hybrids by direct cytotoxic testing. No functional activity was detected in any of the 60 hybrids tested, following either incubation with ^{51}Cr labelled H-2^b tumour target cells, EL-4, or with Con A blasts of H-2^b or H-2^k origin, either in the presence or in the absence of 10 µg/ml Con A as a non-specific 'glue'.

II. Suppression

The results shown here are those accummulated from two separate hybridization experiments. In the first, bacterial contamination wiped out all but two of the hybrids, but both of them proved to be of interest, the supernatants of one of them having antigen specific (KLH) suppressor activity (S1.41) whilst the supernatants of the other (S1.34) were non-specifically suppressive. In a subsequent hybridization 12 more hybrids were grown, of which six had suppressor activity, at least one of which is specific. Data showing the antigen specificity of suppression by S1.34 and S1.41 supernatant materials (SFS1.41 and SFS1.34) are shown in Table 2, in which the activities of SFS1.34 and of SFS1.41 are compared with that of SF prepared from CBA suppressor cells (9) induced by high doses of KLH (100 µg/ml) i.e. the same type of suppressor cells which were used as the normal 'parent' of S1.34 and S1.41. Supernatant material from neither hybrid 'helped' the anti-DNP response when HC were absent.

Table 2. Specificity of suppression by SFS1.34 and SFS1.41

STIMULUS		SUPPRESSION	RESPONSE (d.4, IgM)	Percent of
HC[1)]	Antigen	SF (%)	Anti-DNP AFC/culture ±S.E.	Suppression
-	TNP-KLH	-	10 ± 8	-
HC_{KLH}	"	-	263 ± 19	0
"	"	CBA SF_{KLH} 10	30 ± 10	92
"	"	SFS1.41 10	117 ± 33	58
"	"	" 1	50 ± 5	84
"	"	SFS1.34 10	70 ± 34	76
"	"	" 1	160 ± 16	61
-	"	SFS1.41 10	47 ± 12	-
-	"	SFS1.34 10	25 ± 10	-
-	DNP-(T,G)-A--L	-	156 ± 45	-
$HC_{(T,G)-A--L}$	"	-	453 ± 106	0
"	"	SFS1.41 10	447 ± 98	0
"	"	SFS1.34 10	253 ± 53	67
-	DNP-PAA[2)]	-	4660 ± 821	-
-	"	SFS1.41 10	6413 ± 696	-

1) $3x10^5$ B10.BR HC_{KLH} or $1x10^5$ B10.BR HC_{TGAL} + 10^7 normal B10.BR spleen + 0.1 µg/ml of TNP-KLH or 1 µg/ml DNP-(T,G)-A--L ± SF.

2) 10^7 normal B10.BR spleen + 0.3% final conc. DNP-PAA ± SFS1.41.

The effect of S1.41 on KLH specific help has been tested about 20 times over a period of 13 months with similar results. The specificity of S1.41 suppression has been tested on either GAT or (T,G)-A--L specific help 5 times over a period of 13 months.

The preliminary immunochemical characterization of SFS1.41 is shown in Table 3. The functional molecule(s) was removed not only by antigen (KLH), but also by anti-Ia^k and anti-I-J^k, and the activity could be eluted from the solid phase immunoabsorbents used. In contrast, neither anti-Ia^s nor I-J^s absorbed the activity, and nor did an irrelevant antigen, GAT or rabbit-anti-mouse immunoglobulin (data not shown). The presence of rabbit anti-suppressor factor antiserum and mouse anti-suppressor factor antiserum in the cultures abrogated the suppressive effect of SFS1.41.

Table 3. Molecular characteristics of SFS1.41

STIMULUS		SUPPRESSION	RESPONSE (d.4, IgM)	Percent of
HC[1]	Antigen	SF (10%)	Anti-DNP AFC/culture ± S.E.	Suppression
-	TNP-KLH	-	0	-
B10.HC_{KLH}	"	-	193 ± 40	0
"	"	SFS1.41	3 ± 3	98
"	"	" abs KLH	270 ± 8	0
"	"	" elu "	13 ± 11	92
"	"	" abs α Ia^k	210 ± 5	0
"	"	" elu "	0	100
"	"	" abs α I-J^k	70 ± 38	64
"	"	" elu "	0	100
"	"	" abs α I-J^s	7 ± 6	96
"	"	" elu "	193 ± 7	0
"	"	$CBASF_{KLH}$	0	100
-	TNP-KLH	-	470 ± 56	-
$CBAHC_{KLH}$	"	-	1630 ± 78	0
"	"	SFS1.41	973 ± 48	57
"	"	" +M α SF[2]	1463 ± 385	14
"	"	" +R α SF[2]	1823 ± 94	0

1) $3x10^5$ HC + 10^7 normal B10 spleen + 0.1 μg/ml of TNP-KLH ± S1.41. Two other experiments gave similar results.

2) Rabbit anti-$CBASF_{KLH}$ (R α SF) and CBA α $CBASF_{KLH}$ (M α SF) were added to cultures at 0.1% final concentration together with S1.41 or S1.34. Two other experiments gave similar results.

The cell surface markers of selected suppressor hybrids were interesting and not altogether expected (Table 1). Firstly, by no means all of them expressed both Thy 1.1 and Thy 1.2 - 10 of the 13 tested were only Thy 1.1 positive: however, both S1.34 and S1.41 were Thy 1.1 and Thy 1.2 positive, and in view of the more detailed analysis of their functions, quantitative absorptions were carried out with these two cell lines, and with BW, to determine their Ly and Ia phenotypes (Table 1). Neither BW, nor either of these two hybrids were Ia positive using this test with ATH anti ATL antiserum. S1.34, the non-specific suppressor line, was neither Ly 1.1 (CBA genotype) Ly 1.2 (AKR genotype) nor Ly 2.1 (CBA and AKR genotype) positive, and nor did our sub-line of BW express any of these markers, in contrast to a report that it is Ly 1 positive (13). In contrast S1.41, the specific suppressor line, was Ly 1.1 and Ly 1.2 positive, thus expressing both parental alleles for this locus. The finding of an Ly 1^+2^- phenotype for S1.41 was unexpected, since KLH specific suppressor cells induced from normal spleens are Ly 1^-2^+

There remains the possibility that as we phenotyped an uncloned hybrid line of S1.41, it contains more than one population of cells, but this seems unlikely since it has now been growing continuously for 13 months, without any alteration in function of the antigen specific suppressor supernatant material.

III. Help

The fusion performed with *in vitro* induced (T,G)-A--L specific helper cells is a recent one, and although 19 lines have been established of which 15 have been tested for function, there is no specificity data on whether the helper function displayed by any of them is antigen specific, but 12 gave supernatants which showed the same degree of help as 'conventional' (T,G)-A--L helper factor (14): 3 of these have been cloned producing 49 clones altogether, and in each case more than 50% of the clones of each hybrid have shown help for anti-DNP responses stimulated by DNP-(T,G)-A--L.

D. DISCUSSION

T-T cell hybrids, expressing T cell surface marker characteristics of both parental cell types are not difficult to obtain using BW as the T cell tumour line, and polyethylene glycol as the fusing agent. Approximately 1 cell per 5×10^5 *in vitro* primed T cells fuses with BW to produce a hybrid. Detecting function in such hybrids appears not to be easy in the case of cytotoxicity. There are possible explanations for this, although the negative findings do not prove the widely discussed hypothesis that cytotoxic cells kill the tumour parent, and so prevent formation of fusion hybrids (4,15).

In the experiments reported here, the 'rescue' and perpetuation of specific suppressor function is very much better established than that of helper function, where we have no specificity data at the moment. The need to examine each supernatant for functional activity in a fairly complicated biological assay has placed certain restrictions on the speed of progress. Nevertheless our most studied suppressor hybrid, S1.41, appears to be both specific in its action, and stable in as much as it has retained function and cell surface markers for over a year.

Immunochemical analyses of the suppressor factor found in the supernatant of S1.41 indicate that it is similar if not identical to 'conventionally' induced suppressor factor, and the possibility of obtaining very large quantities of this material for further analyses should throw light on the nature of suppressor factor. It is possible that this material may be the T cell receptor(s). Thy 1 phenotyping was initially done early but H-2 and Ly phenotyping was performed only after one year of growth. It may be significant that with the apparently inevitable loss of chromosomes by hybrids with time (13), H-2, Thy 1 and Ly 1 and 6 antigens and therefore the chromosomes coding for these loci appear to be preserved. This may reflect the need for antigens of these loci to be present on the cell surface, in order to permit T cell function.

Acknowledgments. We thank Deirdre Hayton, Pirkko Himberg and Pirjo Ranttila for excellent technical assistance, Dr M. Rittenberg for KLH, Dr P. Maurer for GAT, Dr Edna Mozes for (T,G)-A--L and Dr M. Baltz for DNP beads. These experiments have been supported by the Imperial Cancer Research Fund, USA Public Health Service Grant AI-13145-02, and the Medical Research Council of Great Britain. Sirrka Kontiainen and P. Vogt are supported by EMBO Fellowships.

References

1. Simpson, E., Beverley, P.C.L.: T cell subpopulations. Progress in Immunology III, 206-216 (1977).
2. Woody, J.N.: Ly-6 is a T cell differentiation antigen. Nature 269, 61-63 (1977).
3. Köhler, G., Milstein, C.: Derivation of specific antibody - producing tissue culture and tumour lines by cell fusion. Eur. J. Immunol. 6, 511-519 (1976).
4. Köhler, G., Lefkovits, I., Elliot, B., Coutinho, A.: Derivation of hybrids between a thymoma line and spleen cells activated in a mixed leucocyte reaction. Eur. J. Immunol. 7, 758-761 (1977).
5. Kontiainen, S., Feldmann, M.: Suppressor cell induction *in vitro*. IV. Target of antigen specific suppressor factor and its genetic relationships. J. Exp. Med. 147, 110-127 (1978).
6. Kontiainen, S., Feldmann, M.: Suppressor cell induction *in vitro*. I. Kinetics of induction of antigen specific suppressor cells. Eur. J. Immunol. 6, 296-301 (1976).
7. Kontiainen, S., Feldmann, M. Induction of specific helper cells *in vitro*. Nature 245, 285-286 (1973).
8. Pontecorvo, G.: Production of mammalian somatic cell hybrids by means of polyethylene glycol treatment. Somat. Cell Genet. 1, 397-400 (1975).
9. Kontiainen, S., Feldmann, M. Suppressor cell induction *in vitro*. III. Antigen specific suppression by supernatants of suppressor cells. Eur. J. Immunol. 7, 310-314 (1977).
10. March, S.C., Parikh, I., Cuatre Casas, P.: A simplified method for cyanogen bromide activation of agarose for affinity chromatography. Analyt. Biochem. 60, 149-152 (1974).
11. Kontiainen, S., Feldmann, M. Suppressor cell induction *in vitro*. V. Effects of anti-suppressor factor preparations. Submitted.
12. Goldsby, R.A., Osborne, B.A., Simpson, E., Herzenberg, L.A.: Hybrid cell lines with T cell characteristics. Nature 267, 707-708 (1977).
13. Hammerling, G.J.: T lymphocyte tissue culture lines produced by cell hybridization. Eur. J. Immunol. 7, 743-746 (1977).
14. Howie, S., Feldmann, M.: *In vitro* studies on H-2 linked unresponsiveness to synthetic polypeptides. III. Production of an antigen specific T helper cell factor to (T,G)-A--L. Eur. J. Immunol. 7, 417-421 (1977).