Immunochemical Properties of Antigen-Specific Monkey T-Cell Suppressor Factor Induced with a *Streptococcus mutans* Antigen

JONATHAN R. LAMB,¹ EDWARD D. ZANDERS,^{1†} SIRKKA KONTIAINEN,² AND THOMAS LEHNER¹

Department of Oral Immunology and Microbiology, Guy's Hospital Medical and Dental School, London SE1 9RT, England,¹ and Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, Finland²

Antigen-specific suppressor factor could be released from monkey suppressor T cells induced in vitro with a protein antigen isolated from the cariogenic bacterium *Streptococcus mutans*. The suppressor activity was due to the factor itself and not to carryover of free antigen. Characterization of the monkey factor revealed it to have a molecular weight of ca. 70,000, and to contain a constant region and determinants encoded by the major histocompatibility complex. The presence of immunoglobulin determinants could not be demonstrated. However, by virtue of its adsorption to specific antigen, an antigen-combining site was shown to be present. The possible regulatory role of streptococcal antigen-specific suppressor factor in protection against dental caries is discussed.

Immunization with *Streptococcus mutans* can elicit protection against dental caries, but can also fail to achieve a protective immune response. Failure to protectively immunize with cells or cell walls of *S. mutans* has been ascribed to a lack of induction of salivary antibodies (30) to a delay in the appearance of the immunoglobulin G (IgG) class of antibodies (3, 17, 18) and to a low IgG/IgA antibody ratio (18). In addition to the requirement of protective antibodies, an effective cell-mediated immune response seems also necessary (18, 19). As suppressor cells and factors can be induced in vitro by cell wall-associated protein antigens of *S. mutans* (15), another possible reason for the lack of protection against dental caries is the development of suppressor activity.

The present studies were carried out to determine the immunochemical characteristics of T-cell suppressor factor derived from rhesus monkey peripheral blood lymphocytes (PBL) induced with a protein antigen from *S. mutans* as previously described (15). The functional suppressor factor was characterized in terms of its molecular weight, antigen-combining site, lack of immunoglobulin determinants, factor constant region, and the presence of Ia antigen determinants.

MATERIALS AND METHODS

Animals. B10.BR mice of both sexes, age 3 to 6 months, bred at the Imperial Cancer Research Fund,

† Present address: ICRF Human Tumour Immunology Group, University College Hospital Medical School, London WC1E 6JJ.

London, England, were used as a source of spleen cells. Rhesus monkeys (*Macaca mulatta*) maintained at Guy's Hospital and bled from the femoral vein provided a source of PBL.

Antigens. Streptococcal antigen I/II (SAI/II) was prepared from *S. mutans* serotype c (Guy's strain) by diethylaminoethyl-cellulose chromatography followed by gel filtration on Sepharose B as described previously (21, 23). Keyhole limpet hemocyanin (KLH) was the generous gift of M. Rittenberg, Portland, Ore. Dinitrophenylated SAI/II (DNP-SAI/II) and trinitrophenylated KLH (TNP-KLH) had five and eight groups of DNP and TNP per 100,000 daltons, respectively. TNP-KLH was a gift of Marilyn Baltz, and DNP-SAI/II was prepared by using dinitrofluorobenzene (16). The latter was compared with unhaptenated antigen by immunodiffusion in agarose against rabbit antiserum to SAI/II prepared as described below. A line of total identity was observed.

Antisera. Rabbit anti-rhesus monkey IgM, IgG, and IgA were purchased from Nordic Diagnostic, Ltd., Maidenhead, Berkshire, England. Their specificities were checked by immunoprecipitation with the corresponding antigens. The antiserum to SAI/II was raised in rabbits as described elsewhere (E. D. Zanders, J. R. Lamb, S. Kontiainen, and T. Lehner, Immunology, in press). Briefly, NZB rabbits were injected intramuscularly with 100 µg of SAI/II in Freund complete adjuvant followed 1 month later with subcutaneous injection of a further 100 µg in incomplete adjuvant. A crude immunoglobulin fraction was obtained by two successive precipitations with 50% ammonium sulfate. Specificity was established by immunodiffusion against purified antigens. Rabbit antisera to KLH specific helper and suppressor factor of mouse (CBA) origin were prepared in Helsinki as previously described (12). The monoclonal DR common antiserum (DA2) was kindly prepared and do-

nated by M. Crumpton, Imperial Cancer Research Fund, London (1).

Preparation and assay of suppressor factors. The optimal conditions for the production and assay of monkey suppressor factor have been established previously (15) by using the Marbrook-Diener culture system described by Kontiainen and Feldmann (10). Rhesus monkey PBL were obtained from heparinized blood by centrifugation through Ficoll-Hypaque density gradients. *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered minimal essential medium or RPMI 1640 medium (Gibco BioCult, Paisley, Scotland) was used throughout. After the PBL were washed through a layer of fetal calf serum, they were used at a concentration of 5×10^6 in 1 ml of medium containing 5% fetal calf serum. These were cultured with 100 μ g of SAI/II in the insert of a modified Marbrook flask. The outer chamber was filled with minimal essential medium or RPMI 1640 buffered with bicarbonate and containing 5% newborn calf serum. After 4 days at 37°C in a humidified atmosphere of 10% CO₂ in air, the suppressor cells were harvested, washed once, and recultured with 0.1 μ g of SAI/II at 5×10^6 viable cells per ml as before. After 24 h, the cells were pelleted and the resulting supernatant, termed suppressor factor, was stored at -20°C before use. This was assayed in terms of its ability to suppress the response of mouse helper cells during the production of specific antibody (10). Mouse helper cells were induced by culturing 15×10^6 unprimed B10.BR spleen cells with 0.01 μ g of SAI/II in the inner chamber of Marbrook flasks for 4 days exactly as described above for monkey suppressor cells, except that the outer chamber contained 5×10^{-5} M mercaptoethanol. Cooperative cultures were established using 3×10^6 helper cells, 10×10^6 unprimed B10.BR spleen cells, and 0.1 μ g of DNP SAI/II in 1 ml of medium containing 5% FCS and 5×10^{-5} M mercaptoethanol. Suppressor factor was added to a 10% final concentration, and triplicate cultures were incubated in Marbrook flasks for 4 days as described above. After the cultures were harvested and washed once in HEPES-buffered medium, specific antibody-forming cells (AFC) were detected by the modified Cunningham assay (2) by using DNP Fab-coated sheep erythrocytes as indicator cells (9). Since unprimed mice were used, only primary anti-DNP responses due to IgM class antibody were detected. Results were expressed as the number of AFC $\pm$ the standard error of triplicate cultures. Suppression of the helper cells directed against SAI/II in an anti-DNP response was expressed as before (11): % suppression = $100 - [(suppression - background)/(help - background)] \times 100$. Background values were obtained by culturing DNP-SAI/II in cooperative cultures with B10.BR spleen cells in the absence of helper cells.

Radioiodination of SAI/II. A 375- μ g amount of SAI/II was iodinated by the chloramine T method (6) by using 1 mCi of ¹²⁵I (Radiochemical Centre, Amersham). After removal of free iodine, the specific activity was 2×10^7 cpm/ μ g.

Gel exclusion chromatography. Suppressor factor was applied to a column (2 by 90 cm) of Sephadex G-75 (Pharmacia Ltd., Hounslow, Middlesex) precali-

brated with 10 mg each of human serum albumin (Calbiochem, Bishops Cleeve, Herts), ovalbumin (Sigma Ltd., Poole, Dorset), and lysozyme (Worthington Biochemicals Corp., Freehold, N.J.). The void volume was determined with blue dextran (Pharmacia). After elution at 20 ml/h in phosphate-buffered saline, 3-ml fractions were collected. Those fractions encompassing the void volume and ovalbumin (45,000 daltons) were assayed in the cooperative culture at a final dilution of 10%. The remaining fractions were pooled and assayed for suppressor activity at the same dilution.

Preparation and use of immunoadsorbents. Antigens and antisera were coupled to Sepharose 4B activated with cyanogen bromide (Pharmacia) according to the manufacturer's instructions. Purified proteins were coupled at 1 mg/ml of adsorbent, and antisera were coupled at 100 μ l of whole serum per ml. Before use the adsorbents were washed once with 3 M sodium thiocyanate followed by phosphate-buffered saline (PBS). A 0.5-ml amount of suppressor factor was mixed batchwise with 1 ml of each adsorbent at 4°C for 1 h. The volume of beads was chosen so as to provide an excess of reactive groups since 100 μ l was sufficient to bind suppressive activity in pilot experiments. Material which did not adsorb specifically to the immobilized antigens or antisera was collected by centrifugation, and the latter washed three times in phosphate-buffered saline. Suppressive activity specifically bound to these adsorbents was eluted with 0.5 ml of 3 M NaSCN and dialyzed overnight against saline at 4°C. Both unbound and specifically bound (NaSCN eluted) material was filtered through membrane filters (Millipore Corp., Bedford, Mass.) and stored at -20°C before use. A 3-ml column of SAI/II Sepharose was employed for the ¹²⁵I carryover experiment. Fractions of 2 ml were collected at a flow rate of 10 ml/h, and the ¹²⁵I radioactivity was monitored in a gamma counter. Peak fractions of unbound and NaSCN eluted material were assayed for suppressive activity.

RESULTS

Antigen carryover of suppressor factor. To exclude the possibility that suppression in this system is due to carryover of antigen by cells or factors, ¹²⁵I-labeled SAI/II was used as stimulating antigen. Monkey PBL were cultured as described in Materials and Methods and harvested after 4 days, and the amount of residual antigen was estimated by gamma counting. After a further 24-h culturing with 0.1 μ g of ¹²⁵I-labeled SAI/II, suppressor factor was collected and counted. Total of 3.8 and 14 ng of antigen were associated with suppressor cells and supernatants, respectively. The latter was further fractionated on a column of SAI/II coupled to Sepharose. After removal of unadsorbed material, 65 pg of antigen was eluted with 3 M NaSCN (data not shown). This eluate was further fractionated by Sephadex G-75 gel filtration (Fig. 1). Suppressive activity coeluted with human serum

FIG. 1. Antigen carryover of suppressor factor. Monkey PBL suppressor cells and factor were induced with ^{125}I -labeled SAI/II. After passage of the factor through a 3-ml column of SAI/II Sepharose, specifically adsorbed material was eluted with 6 ml of 3 M NaSCN and counted. The bulk of this eluate was dialyzed against saline and fractionated into 3-ml fractions on a column of Sephadex G-75. (A) Suppression of anti-DNP responses: 3×10^5 helper cells and 10×10^6 unprimed B10.BR spleen cells were cultured with 0.1 μg of SAI/II in cooperative cultures containing each fraction at a 10% concentration. AFC were assayed from triplicate cultures and expressed as $\text{AFC} \pm \text{standard error}$. HC, Response of helper cells and spleen cells in the absence of suppressor factor; MSF, suppression of helper cell response by unfractionated monkey suppressor factor; BG, background AFC produced by B10.BR spleen cells + 0.1 μg of DNP-SAI/II; HSA, elution position of human serum albumin. (B) ^{125}I radioactivity profile.

albumin at a point where no radioactivity was observed. We conclude, therefore, that antigen associated with suppressor factor is insufficient to suppress per se, since greater than 10 μg is required to suppress helper cell responses (Table 1).

Adsorption of suppressor factor on im-

TABLE 1. Suppressive effects of free antigen

Helper cells	Stimulus ^a		Response	
	Antigen	Suppression ^b	AFC/culture $\pm$ SE ^c	% Suppression
—	DNP-SAI/II ^d	— ^e	57 $\pm$ 15	—
HC _{SAI/II}	+	—	547 $\pm$ 78	0
+	+	0.01 μg SAI/II	497 $\pm$ 94	10
+	+	0.1	580 $\pm$ 67	0
+	+	1.0	477 $\pm$ 52	14
+	+	10	93 $\pm$ 18 ^f	93
—	—	—	—	—

^a 3×10^5 B10.BR HC_{SAI/II} were added to 10×10^6 unprimed B10.BR spleen cells and 0.1 μg of DNP SAI/II. AFC per culture were assayed on day 4.

^b Free SAI/II was added at the initiation of the cooperative culture.

^c SE, Standard error.

^d Background response of 10×10^6 unprimed B10.BR spleen cells to 0.1 μg of DNP-SAI/II.

^e —, No stimulus.

^f Response of 10×10^6 unprimed B10.BR spleen cells without added antigen. Student's *t* test was used to assess the significance of the suppressor effects compared with the helper response. $n = 6$; $P < 0.01$.

munoadsorbents. Monkey SAI/II-induced suppressor factor showed specificity in binding to SAI/II but not to KLH immunosorbents (Fig. 2). This presumably reflects the presence of a specific binding site for antigen on suppressor factor. Although definitive evidence for such idiotypic determinants is lacking in our system, Germain et al. have described shared idiotypes between a suppressor factor to D-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) and B cells in mice (4). Anti-SAI/II adsorbed out the activity of suppressor factor, suggesting that antigenic determinants might be included in suppressor factor preparations (Fig. 2).

The presence of immunoglobulin determinants was tested for by using rabbit anti-rhesus monkey IgM, IgG, and IgA as immunoadsorbents. There was, however, no significant binding of suppressor factor activity to any of these antisera bound to adsorbents (Fig. 3), implying that classical immunoglobulin determinants are not present in suppressor factor.

The activity of monkey SAI/II-induced suppressor factor could be adsorbed by rabbit anti-suppressor factor preparations raised against mouse antigen-specific suppressor factors (CBA.SF_{KLH}), but not by rabbit anti-helper factor preparations raised against mouse antigen-specific helper factors (Fig. 4). These results suggest that one functional class of T-cell factors, antigen-specific suppressor factor of monkey and mouse origin, have shared (or cross-reactive) determinants.

The presence of determinants encoded by the major histocompatibility gene complex (MHC)

FIG. 2. Adsorption of suppressor factor to Sepharose 4B-insolubilized SAI/II and KLH. A 0.5-ml amount of monkey suppressor factor was mixed for 1 h at 4°C with 1 ml of SAI/II or KLH coupled to Sepharose 4B at 1 mg/ml. Material not bound to the adsorbents (unshaded histograms) was separated by centrifugation, and after three washes with phosphate-buffered saline specifically bound material was eluted from the beads with 3 M NaSCN (shaded histograms). Both fractions were assayed in cooperative cultures as described in the text at a 10% final concentration. HC, MSF, and BG were controls as detailed in the legend to Fig. 1. Student's *t* test was used to assess the significance of the suppressor response compared to the helper response alone. *L* = 6; *B* = *P* < 0.05.

FIG. 3. Reaction of suppressor factor with anti-immunoglobulin antisera. Immobilized on Sepharose 4B at 100 μ l/ml. Anti-IgM, anti-IgG, and anti-IgA were insolubilized on Sepharose 4B. After adsorption and elution as described in the legend to Fig. 2, fractions were added at a final concentration of 10% to cooperative cultures as before. AFC were assayed on day 4. For controls and *P* values, see legend to Fig. 2.

in monkey suppressor factor was suggested by the adsorption of suppressor factor to a monoclonal antiserum raised against a common portion of the human HLA-DR gene product (Fig. 4).

DISCUSSION

Antigen-specific T-cell factors capable of suppressing antibody responses have been extensively reported (10, 26, 33). They have been

isolated mostly as cell extracts from in vivo-induced antigen-specific suppressor cells (3, 26). However, monkey antigen-specific suppressor factor was released into the supernatants of suppressor cell cultures (15). This is similar to the original findings reported in mice (10, 11) and more recently in humans (33) using in vitro-induced suppressor cells.

Monkey antigen-specific suppressor factor has an antigen-combining site which is specific for

FIG. 4. Identification of constant region and major histocompatibility complex-encoded determinants in suppressor factor. Rabbit antisera to mouse helper and suppressor factors, as well as a monoclonal antiserum to the common portion of HLA-DR antigens, were coupled to Sepharose 4B and incubated with suppressor factor as described in the text and the legend to Fig. 1. The conditions of assay and controls were as before.

the inducing antigen, SAI/II, derived from *S. mutans* serotype c. This agrees with the specificity of suppressor factors previously described, whether secreted by (14) or extracted from (26) suppressor cells. Similar reports have been made with human suppressor factors (33) (M. Feldmann and S. Kontiainen, manuscript in preparation). All these reports suggest the presence of an antigen-combining site in specific suppressor factors. Recently, Kontiainen and Feldmann reported that syngeneic mouse antiserum to mouse antigen-specific suppressor factor (CBA anti-CBA.SF_{KLH}) showed strict antigen and strain specificity, suggesting that they recognize the variable region (idiotype) of suppressor factors. This was confirmed by Germain et al. (4), who demonstrated that GAT-specific suppressor extracts bear idiotypic markers similar to those found on GAT antibodies. Thus, antigen-specific suppressor factors seem to bear variable region (V) determinants.

Immunoglobulin determinants have not been demonstrated in either suppressor extracts or factors (4, 7, 27, 32; M. Feldmann, P. Erb, S. Kontiainen, I. Todd, and J. M. Woody, *Ann. New York Acad. Sci.*, in press). Further support for the lack of immunoglobulin determinants come from the functional T-cell hybrids secreting antigen-specific suppressor factors (14, 29, 31). The finding that the activity of SAI/II-specific suppressor activity was not adsorbed by rabbit antisera to rhesus monkey IgM, IgG, and IgA agrees with previous findings. However, this is in contrast to our previous report that SAI/II-induced monkey helper factor bound to rabbit anti-rhesus monkey IgM (E. D. Zanders et al., in press). This may imply that determi-

nants cross-reacting with mu chains, or possibly with lymphocyte surface carbohydrates (20) may be present in antigen-specific helper factor but not suppressor factors of monkey origin.

Since SAI/II-specific monkey suppressor factor bound to insolubilized rabbit anti-SAI/II, it appears that a fragment of antigen is present in the factor. However, this may only be loosely associated with suppressor factor since radioiodinated suppressor factor, after a two-step purification on an affinity column and gel filtration, contained no detectable antigen. Nevertheless, suppressor factor still retained functional activity, unlike GAT suppressor extract, which after purification on insolubilized GAT and anti-I-J antiserum could only function when nanogram amounts of free antigen (GAT) are added to the extract. However, GAT-specific suppressor extracts act at a different point in that they induce more suppressor cells from suppressor cell precursors, while secreted suppressor factors act on helper cells of the same antigenic specificity as their targets. It is possible, however, that the SAI/II-induced suppressor factor contained antigen after purification but that this was not detectable by radioiodination due to a paucity of tyrosine molecules in antigen fragments. Nevertheless, the amount of antigen even in unpurified suppressor factor (1.4×10^{-2} $\mu\text{g/ml}$) is by itself ca. 1/10,000 of the amount required to suppress the response by 3×10^5 helper cells, since 10 μg of free antigen is required.

The active fraction of suppressor factor coeluted with human serum albumin on gel filtration, suggesting a molecular weight of ca. 70,000, which is similar to that of SAI/II-induced helper factor (E. D. Zanders et al., in press). The mo-

molecular weight of mouse KLH suppressor factor is in the range 50,000 to 70,000 (14). In contrast, the reported molecular weights of the suppressor extracts were in the range of 30,000 to 60,000 (27, 28, 32).

Antisera generated in rabbits against helper or suppressor factors are able to distinguish between the two functional classes of T-cell-derived factors (helper and suppressor factor), but do not discriminate between antigenic specificities of factors or strain of origin (12). The rabbit CBA.SF_{KLH} antiserum adsorbed the activity of monkey SAI/II-specific suppressor factor, unlike the CBA.HF_{KLH} antiserum. A similar pattern of adsorption was observed with mouse (B10.BR) SAI/II-specific suppressor factor (J. R. Lamb, E. D. Zanders, and S. Kontiainen, unpublished data). On this evidence it would appear that there are constant region determinants present in suppressor factors independent of the species of origin, suggesting that there is significant evolutionary conservation.

In both suppressor factors and extracts the presence of Ia determinants encoded by the I-J subregion of the H-2 histocompatibility complex in mice has been widely reported (11, 13, 25). Similarly, it was observed that monkey SAI/II-specific suppressor factor contained determinants analogous to murine Ia antigens, since its activity was adsorbed with a monoclonal antiserum to human HLA-DR (common) (1). In view of the molecular weight of suppressor factor (70,000) it seems unlikely that the Ia determinants of suppressor factor are the two chain B-cell Ia antigens (58,000). The function of Ia determinants in suppressor factors is not known but cannot be solely for genetic restriction, as reported in extracted materials, since suppressor factor acts across the species barrier. Recently, UytdeHaag et al. (33) have described two classes of human antigen-specific suppressor factors, only one of which is genetically restricted in its action.

In protection against dental caries immunization with *S. mutans* has sometimes failed. One explanation may be that under those conditions suppressor activity has been induced. In vitro induction of suppressor activity with an antigen of *S. mutans* has been described and has been observed to be mediated by a soluble T-cell factor (15). In this report we have described some of the physicochemical properties of the factor, and such a regulatory molecule may influence the outcome of protection against dental caries after vaccination with *S. mutans*.

ACKNOWLEDGMENTS

We thank Marilyn Baltz, M. Crumpton, and M. Rittenberg for the gifts of antigens and antisera. The skillful technical

assistance of Janet Avery and Carol Robinson is gratefully acknowledged. Our thanks also go to Marc Feldmann for helpful discussions.

Jonathan Lamb is in receipt of a Medical Research Council Training Fellowship. This project was carried out under a Medical Research Council project grant.

LITERATURE CITED

1. Brodsky, F. M., P. Parham, C. J. Barnstable, M. J. Crumpton, and W. F. Bodmer. 1979. Monoclonal antibodies for the analysis of the HLA system. *Immunol. Rev.* 47:3-61.
2. Cunningham, A., and A. Szenberg. 1968. Further improvements in plaque techniques for detecting single antibody forming cells. *Immunology* 14:599-601.
3. Germain, R. N., and B. Benacerraf. 1978. Antigen-specific T cell mediated suppression. III. Induction of antigen specific suppressor T cells (TS₂ in L-glutamine acid³⁵-L-alanine³⁰-tyrosine¹⁰) (GAT) responder mice by non-responder derived GAT-suppressor factor (GAT-T,F). *J. Immunol.* 121:608-612.
4. Germain, R. N., S.-T. Ju, T. J. Kippes, B. Benacerraf, and M. Dorf. 1979. Shared idiotypic determinants on antibodies and T cell derived suppressor factor specific for the random terpolymer L-glutamic acid³⁵-L-alanine³⁰-L-tyrosine¹⁰. *J. Exp. Med.* 149:613-622.
5. Germain, R. N., J. Theze, J. A. Kapp, and B. Benacerraf. 1978. Antigen specific T cell mediated suppression. I. Induction of GAT specific suppressor T cells *in vitro* requires both antigen specific T cell suppressor factor and antigen. *J. Exp. Med.* 147:123-136.
6. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine 131 labelled human growth hormone of high specific activity. *Nature (London)* 194:495-496.
7. Kapp, J. A., C. W. Pierce, J. Theze, and B. Benacerraf. 1978. Expression of Ir genes in T cells, p. 529-537. In H. O. McDevitt (ed.), *Ir genes and Ia antigens*. Academic Press, Inc., New York.
8. Kontiainen, S., and M. Feldmann. 1973. Induction of specific helper cells *in vitro*. *Nature (London) New Biol.* 245:285-286.
9. Kontiainen, S., and M. Feldmann. 1975. Conditions for inducing T helper cells *in vitro*. *Scand. J. Immunol.* 4: 121-128.
10. Kontiainen, S., and M. Feldmann. 1977. Suppressor cell induction *in vitro*. III. Antigen specific suppression by supernatants of suppressor cells. *Eur. J. Immunol.* 7:310-314.
11. Kontiainen, S., and M. Feldmann. 1978. Suppressor cell induction *in vitro*. IV. Target of antigen specific suppressor factor and its genetic relationship. *J. Exp. Med.* 147:110-123.
12. Kontiainen, S., and M. Feldmann. 1979. Structural characteristics of antigen specific suppressor factors: definition of "constant" and "variable" region determinants. *Thymus* 1:59-79.
13. Kontiainen, S., S. Howie, P. H. Maures, and M. Feldmann. 1979. Suppressor cell induction *in vitro*. VI. Production of suppressor cells to synthetic polypeptides GAT and (TG)-A-L from cells of responder and non-responder mice. *J. Immunol.* 122:253-259.
14. Kontiainen, S., E. Simpson, E. Bohrer, P. C. L. Beverley, L. A. Herzenberg, W. C. Fitzpatrick, P. Vogt, A. Torano, I. F. C. McKenzie, and M. Feldmann. 1978. T cell lines producing antigen-specific suppressor factor. *Nature (London)* 274:477-480.
15. Lamb, J. R., S. Kontiainen, and T. Lehner. 1979. Generation of specific T-cell suppressor function induced by *Streptococcus mutans* in monkeys and mice. *Infect. Immun.* 26:903-909.
16. Lamb, J. R., S. Kontiainen, and T. Lehner. 1980. A comparative investigation of the generation of specific T cell-helper function induced by *Streptococcus mutans*

- in monkeys and mice. *J. Immunol.* **124**:2384-2389.
17. Lehner, T., S. J. Challacombe, and J. Caldwell. 1976. Immunologic basis for vaccination against dental caries. *J. Dent. Res.* **55**:C166-180.
 18. Lehner, T., M. W. Russell, C. M. Scully, S. J. Challacombe, and J. Caldwell. 1979. The Role of IgG, IgA and IgM classes of antibodies to *Streptococcus mutans* in protection against caries in rhesus monkeys, p. 215-217. In M. T. Parker (ed.), *Pathogenic streptococci*. Reedbooks, Chertsey, Surrey.
 19. Lehner, T., M. W. Russell, J. M. A. Wilton, S. J. Challacombe, C. M. Scully, and J. E. Hawkes. 1978. Passive immunization with antisera to *Streptococcus mutans* in prevention of dental caries in rhesus monkeys. *Adv. Exp. Med. Biol.* **107**:303-315.
 20. Merler, E., J. Gatien, and G. de Wilde. 1974. Significance of immunofluorescent staining of lymphocytes with antisera to IgM immunoglobulins. *Nature (London)* **251**:652-654.
 21. Russell, M. W., L. A. Bergmeier, E. D. Zanders, and T. Lehner. 1980. Protein antigens of *Streptococcus mutans*: purification and properties of a double antigen and its protease-resistant component. *Infect. Immun.* **28**:486-493.
 22. Russell, M. W., S. J. Challacombe, and T. Lehner. 1980. Specificity of antibodies induced by *Streptococcus mutans* during immunization against dental caries. *Immunology* **40**:97-106.
 23. Russell, M. W., and T. Lehner. 1978. Characterization of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype c. *Arch. Oral. Biol.* **23**:7-15.
 24. Tada, T., and M. Taniguchi. 1976. Characterization of antigen specific suppressive T cell factor with special reference to the expression of I region genes, p. 513-534. In D. H. Katz and B. Benacerraf (ed.), *The role of products of the major histocompatibility gene complex in immune responses*. Academic Press, Inc., New York.
 25. Tada, T., M. Taniguchi, and C. S. David. 1977. Suppressive and enhancing T cell factors as I region gene products: properties and the subregion assignment. *Cold Spring Harbor Symp. Quant. Biol.* **41**:119-127.
 26. Tada, T., M. Taniguchi, and T. Takemori. 1975. Properties of primed suppressor T cells and their products. *Transplant. Rev.* **26**:106-129.
 27. Takemori, T., and T. Tada. 1975. Properties of antigen specific T cell suppressor factor in the regulation of antibody responses in the mouse. I. *In vivo* activity and immunochemical characterization. *J. Exp. Med.* **142**:1241-1253.
 28. Taniguchi, M., and J. F. A. P. Miller. 1978. Specific suppression of the immune response by a factor obtained from spleen cells of mice tolerant to human γ globulin. *J. Immunol.* **120**:21-26.
 29. Taniguchi, M., T. Saito, and T. Tada. 1979. Antigen-specific suppressive factor produced by transplantable I-J bearing T-cell hybridoma. *Nature (London)* **278**:555-558.
 30. Tanzer, J. M., G. J. Hageage, and R. H. Larson. 1973. Variable experiences in immunization of rats against *Streptococcus mutans* associated dental caries. *Arch. Oral. Biol.* **18**:1425-1439.
 31. Taussig, M., and A. Holliman. 1979. Structure of an antigen specific factor produced by a hybrid T cell line. *Nature (London)* **277**:308-310.
 32. Theze, J., J. A. Kapp, and B. Benacerraf. 1977. Immunosuppressive factor(s) extracted from lymphoid cells of non-responder mice primed with L-glutamic acid¹⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT). *J. Exp. Med.* **145**:839-856.
 33. UytdeHaag, F., C. J. Heijnen, C. H. Pot, and R. E. Ballieux. 1979. Human B cell activation *in vitro*: regulation by antigen-specific human suppressor T cells, p. 141-157. In A. S. Fauci, and R. E. Ballieux (ed.), *Antibody synthesis in man: in vitro synthesis and clinical implications*. Academic Press, Inc., New York.