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Immunology

Acetylcholine receptor-specific suppressive T-cell factor from a retrovirally transformed T-cell line

(myasthenia gravis/immunoregulation/radiation leukemia virus/antigen-specific T-cell clones)

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ABSTRACT **In both experimental and human myasthenia gravis an impairment in the immune regulation leads to an increased synthesis of antibodies against the nicotinic acetylcholine receptor (AcChoR). The present work reports the establishment of an AcChoR-specific suppressive T-cell line obtained by viral transformation of AcChoR-enriched murine T lymphocytes. Enriched T cells from *Torpedo* AcChoR-primed mice, prestimulated *in vitro* with antigen, were infected with radiation leukemia viruses and injected intravenously in congeneic recipient mice. Six months later lymphomas were observed in 20% of the injected mice and two of them, of donor origin, were established as permanent continuous cell lines *in vitro*. One of these lines, named LA41, expresses Thy-1.2, Lyt-2, and I-J^b surface markers. Culture supernatants of LA41 cells suppress the antigen-specific *in vitro* proliferation of *Torpedo* AcChoR-primed lymphocytes. This suppression is antigen-specific since the response induced by fetal calf AcChoR and by other antigens is not affected by addition of LA41 culture supernatant in the proliferative assay. LA41 culture supernatant injected *in vivo* at the time of antigen-priming suppresses also significantly the production of anti-AcChoR antibodies but not the synthesis of antibodies against other antigens—i.e., fetal calf AcChoR or α-bungarotoxin. These data show that LA41 cells constitutively produce *Torpedo* AcChoR-specific suppressor factor.**

Myasthenia gravis (MG) is an autoimmune disease in which the nicotinic acetylcholine receptor (AcChoR) acts as an autoantigen (1). Impaired immune regulation has been suggested as a possible mechanism leading to an increased synthesis of antibodies against the AcChoR in both human MG and experimental autoimmune MG (EAMG) (2, 3). The pathogenic role of anti-AcChoR antibodies in decreasing the neuromuscular junction AcChoRs has been recently established (3) but the process by which this autoimmune response is triggered is still unknown. A dysfunction in the regulation of T cells could be one of the major causes.

The role of T cells in the pathogenesis of MG has been suggested by several observations. Peripheral T lymphocytes of EAMG-affected animals can be prestimulated *in vitro* by AcChoR (4, 5). AcChoR-specific T lymphocytes from EAMG rats have been shown to be involved in immune responses against AcChoR and in the development of EAMG (5, 6). T cells from MG patients proliferate *in vitro* in the presence of AcChoR (7–9) and the analysis of T-cell subsets in myasthenics has shown, in several patients, a defect in peripheral T-suppressor cell number (10, 11) or an impairment of T-cell suppressor functions (10–13).

The possibility of obtaining continuous AcChoR-specific T-cell lines may clarify the role played by different T-cell subsets in the autoimmune response to AcChoR. Several techniques have been developed to obtain antigen-specific functional T-cell clones. By using T-cell growth factor, both human and murine T cells have been cloned (14, 15). Cell fusion of antigen-primed T lymphocytes with lymphoma T cells has also provided functional T-cell lines (16, 17). Nevertheless, the problems encountered with both techniques, such as rapid chromosome segregation, which occurs in T hybridomas, or the loss of functional activity occurring during long-term culture of some T-cell clones, have not yet been solved (18). A different approach, using the transforming radiation leukemia virus (RadLV), which selectively infects T cells, has allowed successful transformation of preselected, antigen-specific functional T lymphocytes (19–21). Using this technique we have established from RadLV-transformed *Torpedo* AcChoR-primed T lymphocytes several T-cell lines and tested the antigen-specific functional activity of their products.

MATERIALS AND METHODS

Antigen Preparation. Fetal calf and *Torpedo californica* AcChoRs were prepared as described (22). Fetal calves were obtained from the local slaughterhouse and *T. californica* were a generous gift of M. Raftery (Caltech, Pasadena, CA). In brief, 200 g of fetal calf muscle or 100 g of *Torpedo* electric organs was homogenized in 2 vol of 50 mM Tris·HCl, pH 7.4/50 mM NaCl/2 mM EDTA/1 mM EGTA/1 mM iodoacetamide/1 mM phenylmethylsulfonyl fluoride (buffer 1). The homogenate was centrifuged for 45 min at 50,000 × *g*. The resulting pellet was rehomogenized in 1 vol of buffer 1 and Triton X-100 was added to a final concentration of 1%. After 2 hr of extraction at 4°C the material was centrifuged at 50,000 × *g* for 50 min. The supernatant was applied to an α-cobra toxin affinity column and then the column was washed extensively. During the washes Triton X-100 was exchanged with Tween 20 or Tween 80 (23). AcChoRs were eluted from the column with 1 M carbamoylcholine in buffer 1. After extensive dialysis against buffer 1 without EDTA, EGTA, iodoacetamide, and 0.1 mM phenylmethylsulfonyl fluoride, the specific activity of AcChoRs measured as nmol of ^{125}I-α-labeled bungarotoxin (^{125}I-α-Bgtx) per mg of protein was found to be 6 for fetal calf AcChoR and 8 for *Torpedo* AcChoR. The recovery from 100 g of wet tissue was 25 μg for fetal calf AcChoR and 5 mg for *Torpedo* AcChoR. α-Bgtx was isolated from the venom of *Bungarus multicinctus* (Sigma) according to Ravdin and Berg (24). The purity of α-Bgtx was assessed by NaDodSO$_4$/polyacrylamide gel electrophoresis.

Mice and Immunization. C57BL/Ka (Thy-1.2$^+$) mice (6–8 weeks old) were injected into the footpad with 100 μl of AcChoR (50 μg/ml) emulsified with an equal volume of complete Freund's adjuvant (CFA) (Difco). As recipients for RadLV-infected T lymphocytes, 6- to 8-week-old C57BL/

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Abbreviations: MG, myasthenia gravis; EAMG, experimental autoimmune MG; RadLV, radiation leukemia virus(es); AcChoR, acetylcholine receptor; α-Bgtx, α-bungarotoxin; CFA, complete Freund's adjuvant; i.v., intravenous(ly).

Ka (Thy-1.1$^+$) mice were used, kindly provided by M. Lieberman and H. S. Kaplan (Stanford University). Prior to cell injection recipient mice were x-irradiated with a dose of 450 rads (1 rad = 0.01 gray).

Enrichment of AcChoR-Specific T Cells. Seven days after priming, popliteal lymph nodes were collected and pooled, and single-cell suspensions were prepared. Lymph node cells (4×10^6 per ml) were cultured for 7 days in 100-mm Petri dishes in RPMI 1640 medium supplemented with 10% fetal calf serum containing 0.2 μg of AcChoR per ml. After 1 week, cells were washed and replated with 20×10^6 irradiated (2000 rads) syngeneic spleen cells in fresh medium and 0.2 μg of AcChoR per ml for 7 additional days. The contaminating B cells were depleted by panning on anti-mouse Ig-coated 100-mm Petri dishes. The proliferation of antigen-specific T cells was monitored by [^{3}H]thymidine incorporation.

Viral Infection. The retrovirus, RadLV strain Nu_1, was harvested from fresh supernatant of chronically infected BALB/Nu_1 cells (25). Enriched T cells (10×10^6) were plated in 0.5 ml of complete medium supplemented with 4 μg of Polybrene (Sigma) per ml and with 0.5 ml of filtered (0.2 μm) BALB/Nu_1 fresh culture supernatant. Incubation was carried out for 1 hr at 37°C in a humidified CO_2/O_2 incubator and the plates were gently swirled every 15 min. T cells were then extensively washed for three times in 50 ml of medium to remove free viral particles and immediately injected intravenously (i.v.) ($1–5 \times 10^6$ cells per mouse) into congeneic irradiated C57BL/Ka (Thy-1.1$^+$) mice.

Establishment of Permanent Cell Lines. Lymphoma cells (10×10^6 cells), prepared as a cell suspension by mincing neoplastic tissue through a fine mesh steel screen, were plated *in vitro* ($4–5 \times 10^6$ cells per ml) in RPMI medium supplemented with 20% fetal calf serum, 25 μM 2-mercaptoethanol, 1 mM L-glutamine, 100 mM penicillin, streptomycin at 100 μg/ml, and gentamicin at 10 μg/ml. In some cases lymphoma cells were injected intraperitoneally into pristane-primed (0.5 ml) syngeneic mice. After 2 weeks the ascitic cells were collected and replated *in vitro* where they were easily adapted to culture growth. After 6 months of continuous growth, cultures were considered established *in vitro* as a cell line and cloned.

Antisera. Monoclonal anti-Lyt-1.2 and anti-Lyt-2.2 antibodies (Becton Dickinson) were used at a 1:50 dilution, whereas anti-Thy-1.2 and anti-Thy-1.1 antibodies (New England Nuclear) were used at a 1:100 dilution. Anti-I-J^b serum (kindly provided by L. Adorini) was used at a 1:20 dilution. Fluorescein-conjugated goat anti-mouse IgG antibodies (Cappel Laboratories) were used at a 1:20 dilution.

Immunofluorescence. T-cell lines were analyzed by indirect immunofluorescence using a fluorescence-activated cell sorter (FACS IV Becton Dickinson). Cells (2×10^6) were resuspended in 20 μl of phosphate-buffered saline solution (Pi/NaCl) containing monoclonal mouse antibodies to Thy-1.1, Thy-1.2, Lyt-1.2, Lyt-2.2, or I-J^b. After 45 min at 0°C, cells were washed and incubated with fluorescinated goat anti-mouse immunoglobulins for 40 min at 0°C. Cells were then washed and fixed with 1% paraformaldehyde in P_i/NaCl prior to FACS IV analysis.

T-Cell Proliferation Assay. The T-cell proliferation assay was performed according to Corradin (26). Briefly, 8 days after immunization the popliteal lymph nodes were pooled and minced and single-cell suspensions (4×10^5 per well) were cultured in flat-bottom microtiter plates (Falcon, Becton Dickinson) in 0.1 ml of Click medium containing 0.5% syngeneic mouse serum, 25 μM 2-mercaptoethanol, 4 mM L-glutamine, 2.5 mM sodium pyruvate, and 50 μg of gentamicin per ml. Cultures then received 0.1 ml of medium alone or 0.5 μg of antigen per ml either in complete Click medium or in filtered culture supernatant from lymphoma cells. Cells were incubated for 5 days at 37°C in 5% CO_2 humid air. Eighteen hours before harvest each culture was pulsed with 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (2 Ci/mmol, Radiochemical Centre) and then collected with an automated harvester (Skatron, Flow Laboratories). Radioactivity on filter discs was measured in a liquid scintillation counter. Results are expressed as the arithmetic mean ± SEM of cpm from quadruplicate cultures.

Antibody Assay. C57BL/Ka mice were footpad primed with 2.5 μg of *Torpedo* AcChoR or fetal calf AcChoR or with 0.5 μg or 0.05 μg of α-Bgtx emulsified in CFA. Mice were injected i.v. for 3 days, starting the day of immunization, with either saline (0.5 ml/day) or culture supernatant (0.5 ml/day) from the LA41 cell line. After 10, 20, and 30 days, mice were bled and the antibody response against the antigens was assayed.

Torpedo AcChoR (1 nM) was labeled with 2 nM ^{125}I-α-Bgtx for 3 hr at room temperature. Each aliquot of the complex ^{125}I-α-Bgtx–AcChoR was incubated with varying amounts of immune serum and adjusted by dilution with normal serum. Samples were incubated overnight at 4°C and the following day goat anti-mouse IgG and 4% polyethylene glycol 6000 were added to precipitate the ^{125}I-α-Bgtx–AcChoR complexes. Two hours afterward the samples were centrifuged, and the pellets were washed twice with P_i/NaCl containing 0.5% Triton X-100 and assayed for radioactivity in a Beckman γ-counter. The amount of antibody was expressed as pmol of ^{125}I-α-Bgtx binding sites precipitated per ml of serum.

Antibodies against α-Bgtx and fetal calf AcChoR were assayed with a sandwich assay reported by Rennard *et al.* (27) with some modifications. The wells of flat-bottom microtiter plates (Dynatech) were filled with 100 μl of polylysine at a concentration of 25 μg/ml and left for 1 hr at room temperature. The wells were then washed three times with P_i/NaCl, refilled with 100 μl of a solution of 2 μg of α-Bgtx per ml or of 1 μg of fetal calf AcChoR per ml in 20 nM carbonate (pH 9.6), and incubated overnight at 4°C. The fluid was removed after 12 hr and the plates were washed three times with P_i/NaCl containing 0.05% Triton X-100 (P_i/NaCl/T). Serial dilutions of test serum were made in P_i/NaCl/T containing 2% bovine serum albumin and added to the wells to a final volume of 100 μl. Wells treated only with P_i/NaCl/T and bovine serum albumin were included in the assay to determine the background. The plates were incubated at 37°C for 1 hr and then washed thoroughly with P_i/NaCl/T. Rabbit anti-mouse IgG and IgM in P_i/NaCl/T and bovine serum albumin was then added and left for 60 min at 37°C. The plates were washed again with P_i/NaCl/T, and protein A-peroxidase (Sigma) at the optimal concentration was added and incubated for 120 min at 28°C. Excess of protein A-peroxidase was washed with P_i/NaCl/T and the wells were refilled with 200 μl of enzyme substrate. The substrate solution for peroxidase assay consisted of 20 ml of 0.01 M citrate (pH 5.25) containing 1 mg of *o*-phenylenediamine dissolved in 200 μl of methanol and 100 μl of 3% hydrogen peroxide. The substrate was incubated for 60 min at 28°C and the reaction was stopped by adding 50 μl of 2 M sulfuric acid.

The degree of substrate catalysis was determined in optical density units at 492 nm by using a microtiter spectrophotometer. The antibody titer was calculated by linear regression analysis of the optical values of serial dilutions of the test serum after subtraction with preimmune mouse serum values.

RESULTS

Establishment of Permanent T-Cell Lines. Enriched AcChoR-specific T cells from popliteal lymph nodes of C57BL/Ka (Thy-1.2$^+$) mice were infected *in vitro* with RadLV and injected into congeneic C57BL/Ka (Thy-1.1$^+$)

Table 1. Surface marker expression on lymphoma cells

Lymphoma	% positive cells detected by FACS IV analysis					
	Thy-1.2	Thy-1.1	Lyt-2.2	Lyt-1.2	I-J^b	sIg
LA41	92	5	66	5	39	5
LA22	94	6	33	32	12	5
LA10	5	90	ND	ND	ND	ND
LA30	3	95	ND	ND	ND	ND
LA50	5	89	ND	ND	ND	ND
LA60	2	91	ND	ND	ND	ND
LA70	4	92	ND	ND	ND	ND

ND, not done; sIg, surface immunoglobulin.

mice. Seven of 35 injected animals developed lymphomas either in the thymus or in the lymph nodes with a latency period of 6–8 months.

Of 7 lymphomas, 2 were shown to be of donor origin by FACS IV analysis using either monoclonal α-Thy-1.1 or monoclonal α-Thy-1.2 antibody (Table 1). These primary tumors were originally localized in the lymph nodes. They were injected intraperitoneally into syngeneic pristane-primed mice in order to obtain ascitic cells, which are more easily adapted to *in vitro* growth. The ascitic cells were subsequently established *in vitro* as continuous cell lines and designated as LA41 and LA22 cells. Established lymphoma cells grew rapidly, with doubling times between 16 and 20 hr. These cell lines were efficiently cloned by limiting dilution on peritoneal macrophage feeder layers and culture supernatants were tested for functional activity. Both the parental line and clones were continuously kept in culture for >6 months without loss of function. Lymphoma cells stored in liquid nitrogen were easily recovered without loss of viability and function.

Analysis of Surface Antigens. LA41 and LA22 cell lines were examined for cell surface expression of immunoglobulin, Thy-1, Lyt, and I-J^b markers by direct or indirect immunofluorescence by using the monoclonal reagents described in *Materials and Methods*. LA41 and LA22 cells exhibit the Thy-1.2 phenotype on nearly all cells and the absence of Thy-1.1 determinant (Table 1), showing the donor cell-type origin of the lymphoma. LA41 cells are Lyt-2.2$^+$, Lyt-1.2$^-$, and I-J^{b+}, whereas the LA22 cell line expresses both the Lyt-1 and Lyt-2 antigens. No phenotypic expression of surface immunoglobulin could be detected.

Suppression of the *in Vitro* Antigen-Specific Proliferative Response. The biological activity of LA41 culture supernatants was tested by the antigen-specific *in vitro* proliferative assay. Lymph node cells from *Torpedo* AcChoR- or fetal calf AcChoR-immunized mice were cultured with the same antigen used for priming. The addition of LA41 supernatant to cultures was able to induce 64% suppression of the *Torpedo* AcChoR-specific proliferative response (Table 2). This suppression was antigen-specific since it did not occur when using a closely related antigen, the fetal calf AcChoR as priming and stimulating antigen (Table 2).

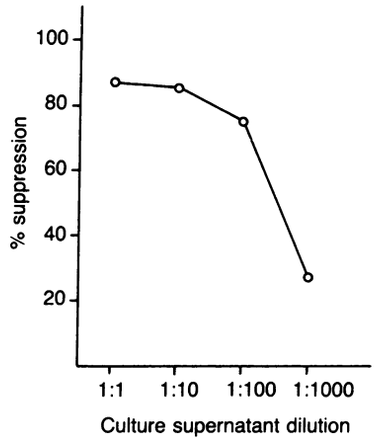

FIG. 1. Titration of AcChoR suppressive activity in LA41 culture supernatants. Supernatant of the LA41 cell line was diluted in culture medium and tested for suppression of AcChoRT-specific proliferative response as described in the legend to Table 2.

To titrate the suppressive activity of LA41 line, culture supernatants were diluted in Click medium and assayed in the *in vitro* proliferative assay. A dilution of 1:100 was still able to give >70% suppression (Fig. 1).

The LA41 cell line was cloned by limiting dilution and culture supernatants from individual clones were tested for suppressive activity of the proliferative response; all of the clones were able to produce the suppressor factor, which suppresses the proliferative response in a range between 60% and 90%. LA22 supernatants, assayed in the same experiments as controls, did not exert a suppressor activity (Fig. 2).

Suppression of the *in Vivo* Antigen-Specific Antibody Response. LA41 supernatant was injected i.v. into C57BL/Ka mice previously primed either with *Torpedo* AcChoRI or with fetal calf AcChoR or α-Bgtx. Mice were bled 10, 20, and 30 days after priming. The specific antibody response against AcChoR was significantly suppressed 10 and 30 days after priming with an inhibition of 65% and 80% at early and late time points, showing that the immune response was not delayed but suppressed at the time of priming. At day 20, suppression, although in the same order of magnitude compared to day 10, was not statistically significant, due to a larger individual variability. Antigen specificity of the suppressive molecule produced by LA41 cells was demonstrated by using the other two antigens, fetal calf AcChoR and α-Bgtx. As shown in Table 3, the immune response against these two antigens was not affected by the injection of LA41 supernatant.

Table 2. *In vitro* fine antigen specificity of LA41 suppressor T-cell factor

LA41 supernatant	Priming *in vivo*	Antigen *in vitro*	cpm	% suppression
−	AcChoRT-CFA	AcChoRT	39,983 ± 2861*	—
+	AcChoRT-CFA	AcChoRT	14,764 ± 2956**	64
−	AcChoRV-CFA	AcChoRV	54,389 ± 412	—
+	AcChoRV-CFA	AcChoRV	53,948 ± 2085	0.8

C57BL/Ka mice were footpad primed with either 2.5 μg of *Torpedo* AcChoR (AcChoRT) or 2.5 μg of fetal calf AcChoR (AcChoRV) in CFA. Seven days later lymph node cells were cultured in the presence of antigen. Culture supernatant from the LA41 cell line, recovered from cells plated in a 100-mm Petri dish at a cell density of 5×10^5 per ml 48 hr prior harvesting, was added at the beginning of the culture and thymidine incorporation was measured after 5 days. Results are expressed as the arithmetic mean of cpm ± SEM from at least five experiments done in quadruplicate cultures. $P < 0.01$ for * vs. **.

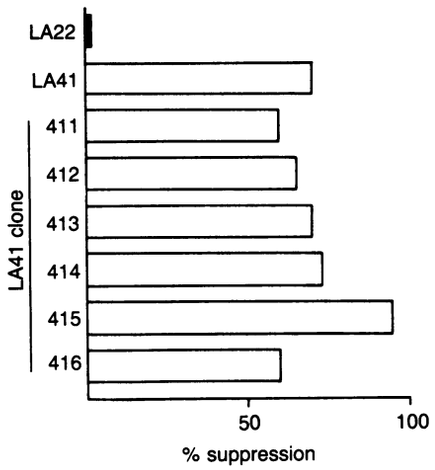

FIG. 2. Suppressive activity of clones derived from the LA41 cell line. Culture supernatants from either the LA22 or LA41 cell line or clones derived from the LA41 line were tested for *in vitro* suppression of AcChoR-specific proliferative response. Lymph node cells (4×10^5) from C57BL/Ka mice immunized with *Torpedo* AcChoR-CFA were cultured with *Torpedo* AcChoR. Culture supernatant (100 μl) from the indicated cell lines and clones was added at the beginning of the culture and thymidine incorporation was measured after 5 days. In this proliferative assay, thymidine incorporation in the AcChoR-stimulated wells was 38,900 ± 1890 cpm. Bars represent % suppression of the AcChoR-specific proliferative response.

DISCUSSION

Antigen-specific suppressor T-cell lines, obtained either by T–T cell fusions (16, 17, 28, 29) or by viral transformation (19–21), have been shown to release in the culture supernatant suppressor factors that interfere with the specific immune response. Immortalization of selected T-cell subpopulations achieved by *in vitro* infection with the RadLV has been a useful method to obtain antigen-specific lymphomas producing immunological active molecules *in vitro* (19, 20). These works have shown that the mechanisms involved in the expression of effector immune functions are not affected by the transformation process (30). The retroviruses used in such experiments belong to the group of "slow" leukemogenic viruses (31). Therefore, *in vitro* virally infected cells become transformed after a long period of time, during which cDNA proviruses integrated into the host genome may randomly rearrange and activate, through a mechanism of viral promoter insertion, the expression of oncogenic (c-*onc*) sequences. Due to the long latency period required to achieve such transformation events, the injection of *in vitro* infected T cells into congeneic hosts is then a convenient procedure.

Through the infection of AcChoR-primed lymphocytes with RadLV we have obtained a cell line (LA41) of T lymphocytes releasing in the culture medium a suppressor factor that is able to induce 80% suppression of the *in vitro* antigen-specific proliferative response. The specificity of the LA41 suppressor molecule was also shown by using culture medium from LA22, a T-cell line similarly obtained, which was unable to induce a specific suppression of the proliferative response. All of the LA41 clones tested displayed the same level of suppressive activity, suggesting that the lymphoma cell was already functionally cloned. The fine antigen specificity exerted by the AcChoR suppressor factor was demonstrated by the fact that it did not suppress the proliferative response against fetal calf muscle AcChoR, a closely related molecule, which has a high degree of homology in the amino acid sequence (32), and common antigenic determinants (33). This result indicates that the specificity of this suppressor factor is restricted to antigenic epitopes present only in the *Torpedo* AcChoR molecule.

The production of anti-AcChoR antibodies in mice injected with LA41 suppressor factor was also significantly and specifically suppressed, as shown by using *Torpedo* AcChoR and α-Bgtx as immunizing antigens, whereas the fine antigen specificity of the suppressor factor was demonstrated by using *Torpedo* and fetal calf muscle AcChoR. *In vivo* suppression of antibody responses can be also induced by nonspecific factors such as viruses that may be present in the supernatant of lymphoma cell lines. In the present report we have shown that the LA41 supernatant is unable to suppress the antibody response to unrelated antigens such as α-Bgtx and fetal calf AcChoR. Moreover, injection of supernatants from the lymphoma cell line RL12, which releases RadLV in the culture medium (34), has been shown to have no effects on the *in vivo* antibody response (35).

The mechanism by which suppression is achieved is still unknown, but helper T cells are likely to be the final cellular target of the suppressor factor either directly or through the activation of different suppressor T-cell subsets (36, 37).

The correlation between phenotype markers and T-cell functions has been suggested by several authors (38, 39). More recently, the use of T-cell hybrids and T-cell lines has allowed a more precise matching between the surface alloantigens and the function of suppressor T cells (20, 40). In our case, the cell line that showed a suppressor activity expressed the Lyt-2 and I-J^b markers, in agreement with re-

Table 3. *In vivo* fine antigen specificity of LA41 suppressor T-cell factor

Antigen	LA41 supernatant	Time of bleeding		
		Day 10	Day 20	Day 30
Torpedo AcChoR, 2.5 μg	−	29.1 ± 7.4*	54.4 ± 17†	118.0 ± 28‡
	+	10.0 ± 4.2*	15.0 ± 9†	21.5 ± 6.9‡
Veal AcChoR, 3 μg	−	—	24.0 ± 3.2‡	68.0 ± 11.4†
	+	—	50.0 ± 5.5‡	81.0 ± 12.6†
α-Bgtx, 0.05 μg	−	—	—	125.0 ± 21†
	+	—	—	178.0 ± 30†
α-Bgtx, 0.5 μg	−	—	—	170.0 ± 15†
	+	—	—	168.0 ± 12†

Statistical analysis was performed with Student's *t* test on values obtained with immunized mice injected with LA41 suppressor factor and mock controls. Eight C57BL/Ka mice per group were injected into the hind footpad with antigens in CFA and bled 10, 20, and 30 days after the immunization. The animals received, from the day of priming, a daily i.v. injection, for 3 days, of 0.5 ml of saline or of LA41 supernatant. Results are expressed as the arithmetic mean ± SEM.

*$P < 0.05$.

†Not significant.

‡$P < 0.01$.

ports on the phenotype of some suppressor T-cell subsets (20, 39). On the basis of surface analysis and of functional activity, we can conclude that LA41 cells have a T-AcChoR-specific suppressor function. Very recently the induction of AcChoR-specific suppressor T cells has been reported in mice (41). Also, in this case, antigen-specific suppression was shown to be mediated by AcChoR-specific factors.

The mechanisms that underlie autoimmunity, and particularly autoimmunity against AcChoR in MG, are multifactorial and are generally unknown. One suggested mechanism that allows induction and/or perpetuation of the autoimmune state is defect or deficiency in suppressor T cells. Reports indicating a decreased function of suppressor T cells in MG patients (12, 13) suggest that the loss of suppressors may be relevant to the pathogenesis of this disease.

The establishment of a suppressor T-cell line specific for AcChoR that constitutively produces a factor that is able to specifically suppress the *in vitro* T-cell proliferation and the *in vivo* antibody response against AcChoR will allow us to study further the regulation and mechanisms involved in autoimmune responses.

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