IL-2 and TNF Receptors as Targets of Regulatory T-T Interactions

Isolation and Characterization of Cytokine Receptor-Reactive T Cell Lines in the Lewis Rat

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T cells are considered to be of prime importance in immune regulation of both B and T cell functions. The targets of recognition in T-T cell interactions are not clear. Most recent experimental work has focused on the idiotypic regulatory interactions mediated by TCR peptides. There is experimental evidence that regulatory cells exist that do not recognize the TCR. This type of regulation is selectively induced by activated T cells. Therefore, we designed this study to examine the possible role of cytokine receptors as targets of immune regulation. We tested two peptides of IL-2R α-chain, 2 of IL-2R β-chain, and one of TNFR (p60). All peptides were found to be immunogenic at inducing T cell proliferation and four induced Abs in Lewis rats. We generated T cell lines to these five peptides, and tested them both in vitro and in vivo. We found that the T cells exhibited a proliferative response when cultured with activated, irradiated stimulator cells that were augmented upon addition of the cytokine receptor peptide. The cytokine profile of the lines was characterized as well as the Vβ gene composition. One of the lines significantly protected against active encephalomyelitis. These results point at cytokine receptors as possible targets of immune regulation and T-T cell interactions. ***The Journal of Immunology,*** **1996, 157: 4855–4861.**

The immune response to foreign invaders is the basis for protective immunity. As the responses of both T and B cells can be quantified, it is clear that in most cases the immune response runs a predictable pattern of induction phase, followed by measurable responses reaching a peak succeeded by a decline in the immune response over time. The basic mechanisms underlying the down-regulation of immune responses are poorly understood, and include: elimination of Ag (1), secretion of suppressive cytokines (2), induction of apoptosis (3, 4), and the emergence of regulatory cells capable of recognizing the proliferating immune cells and inhibiting their responses (5, 6). This type of specific regulation has been termed idiotypic network regulation (7) and has been documented in both B (8) and T cells (9). The targets for regulatory idiotypic T-T interactions have focused much of the recent work on the TCR, and different investigations have yielded results relating the major immunodominant peptides in the complementarity determining region (CDR)1[2] (10), CDR2 (11), CDR3 (12), or framework 3 region (13) of the β-chain of the TCR.

In addition to idiotypically based immune regulation, there is evidence that some type of regulatory cells are induced by activated T cells without relation to the Ag specificity of the cells (14). This type of regulation has been termed anti-ergotypic (14). It has been shown that such regulation could inhibit experimental autoimmune encephalomyelitis (14). As the nature of self Ags recognized by such regulatory cells remained elusive, we designed experiments to test the hypothesis that cytokine receptor peptides could serve as targets for regulatory T-T cell interactions. We chose cytokine receptor peptides since these are surface molecules (likely to be presented by MHC class II molecules) (15), are induced upon T cell activation, and are relatively specific for T cells. While IL-2R γ-chain is constitutively expressed (16), α- and β-chains are induced upon T cell activation. In addition to T cells, IL-2R are expressed by B cells (17) and monocytes (18), and TNFR are expressed by many cell types (19).

We found that in Lewis rats such self-molecules were highly immunogenic both for T and B cells. We generated T cell lines to five peptides from the IL-2 α- and β-chains and from the TNFR, and tested them both in vitro and in vivo. We found that the T cells exhibited proliferative responses when cultured with activated, irradiated stimulator cells that were augmented upon addition of the cytokine receptor peptide. One of the lines significantly protected against active encephalomyelitis. This result points at cytokine receptors as possible targets of immune regulation and T-T cell interactions.

Materials and Methods

Animals

Inbred female Lewis rats were supplied monthly by Harlan Olac, Bicester, U.K. and were used at 2 to 3 mo of age.

Ags and Abs

Myelin basic protein (MBP) from the spinal cords of guinea pigs was prepared as described (20). *Mycobacterium tuberculosis* H37Ra was purchased from Difco, Detroit, MI. Peptides were synthesized using the F-moc technique with an automatic multiple peptide synthesizer (AMS 422, ABIMED, Langenfeld, Germany). The purity of the peptides was analyzed

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[2] Abbreviations used in this paper: CDR, complementarity determining region; MBP, myelin basic protein; EAE, experimental allergic encephalomyelitis; RT, reverse transcriptase; GAPDH, glyceraldehyde phosphate dehydrogenase.

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by HPLC and amino acid composition. mAbs (MRC-ox6 anti-IA, MRC-ox18 anti-IE, anti Vβ 8.2, 8.5, 10, and 16) were purchased from PharMingen, San Diego, CA. FITC-conjugated goat anti-mouse Ab was obtained from Jackson ImmunoResearch, West Grove, PA.

Immunizations

Peptides were dissolved in PBS (2 mg/ml), and oil emulsion was prepared (1:1 ratio) with IFA containing 4 mg/ml *M. tuberculosis* H37Ra. Naive female Lewis rats were immunized in both hind footpads with 50 μl emulsion and each rat was injected with 100 μg of peptide. Draining popliteal lymph node cells were removed on day 12 after injection and a single-cell suspension was prepared by pressing the organs through a fine wire mesh. T cell proliferation assay was performed in 96 microtiter wells (Greiner, Nürtingen, Germany).

T cell proliferation assay

T cell proliferation assay of popliteal lymph node cells from animals primed 12 days previously with the specific peptide in CFA was performed by seeding 2×10^5 cells in stimulation medium for 3 days. Stimulation medium was composed of DMEM supplemented with 2-ME (5×10^{-5} M), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), nonessential amino acids (1 ml/100 ml, BioLab, Jerusalem, Israel), and autologous serum 1% (v/v) (20). The cultures were incubated in quadruplicate for 72 h at 37°C in humidified air containing 7% CO_2. Each well was pulsed with 1 μCi of [^{3}H]thymidine (10 Ci/mmol, sp.act., Nuclear Research, Negev, Israel) for the final 18 h. The cultures were then harvested using a MicroMate 196 Cell Harvester and cpm were determined using a Matrix 96 Direct Beta Counter using avalanche gas (98.7% helium and 1.3% C_4H_{10}) ionization detectors (Packard Instrument Company, Meriden, CT). The results of proliferation are expressed as cpm. In the T cell coculture experiment, clone A2b (21) was used as the stimulator; in the activated form it was irradiated (5000 R) and plated in triplicate in 96-well, round-bottom microtiter plates in twofold dilutions starting from 5×10^4 cells/well and ending with 780 cells/well (1/64 dilution). Clone A2b was chosen because it expresses high levels of MHC class II molecules, and it is activated by IL-2 in the absence of APC (22).

ELISA

Ninety-six-well, flat-bottom microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with the cytokine receptor synthetic peptides (5 μg/ml) by overnight incubation in PBS. After overnight incubation, they were washed with PBS and blocked with 1% Marvell powder milk (Premier Brands, Bourville, Birmingham, U.K.) in PBS for 2 h at 37°C. The plates were then washed with PBS and 100 μl/well of sample serum dilutions in PBS 0.33%-BRIJ 35 (Sigma Chemical Company, Petah Tikva, Israel) and 1% Marvell were added to the plate (two wells per serum dilution). The plates were incubated for 2 h at 37°C and washed in PBS. Next, 100 μl/well alkaline phosphatase-conjugated rabbit anti-rat IgG (Sigma) diluted 1:2500 in PBS 0.33%-BRIJ 35 plus 1% Marvell powder milk were added and the plates incubated for 2 h at 37°C. The plates were washed in PBS 0.33%-BRIJ 35 and in PBS, and 100 μl/well of alkaline phosphatase substrate solution was added. The plates were read with an Anthos HT2 ELISA plate reader, at 405 nm. The OD background values were determined as the mean of the OD values obtained from wells that were coated with the peptides and no serum dilution was added; the OD background were subtracted from the sample sera ODs values.

T cell lines

Ag-specific T cell lines were established from lymph node cells that had been stimulated with peptide (5 μg/ml) for 3 days in stimulation medium as described above. Following stimulation, the T cell blasts were isolated on Lymphoprep (Nycomed Pharma, Oslo, Norway) and seeded in propagation medium (identical to stimulation medium without autologous serum, but supplemented with FCS 10% and T cell growth factors from the supernatant of Con A-stimulated spleen cells 10% (20)). Five days after seeding, the cells (5×10^5/ml) were restimulated with peptide (5 μg/ml) and irradiated thymocytes as APC (10^7/ml) for 3 days in stimulation medium. Lines were expanded by repeated stimulation with peptides and irradiated thymocytes as APC every 10 to 12 days (23). Following four to six rounds of stimulation, the cells were analyzed for their specificity to immunizing peptides in a proliferation assay. When T cell lines reached adequate numbers at the end of a rest phase, 5×10^4 line cells were seeded in 96-well, round-bottom microtiter plates (Greiner, Nurtingen, Germany) with 5×10^5 irradiated (2500 R) thymocytes (23).

Determination of MHC restriction pattern

To determine MHC restrictions of responding T cell lines, anti-IA or anti-IE mAbs (MRC-OX6 or MRC-OX17, respectively, obtained from PharMingen) were added to T cell proliferation wells. In the lines that were IA restricted, addition of the mAb resulted in over 80% inhibition of proliferation relative to control cultures.

Flow cytometry

Line cells were incubated at 4°C for 45 min with anti-Vβ Abs that were obtained from PharMingen. Secondary rabbit anti-mouse FITC-conjugated Abs were incubated at 4°C for 30 min. The cells were then washed and fluorescence was measured using the FACSort (Becton Dickinson, Mountain View, CA). Analysis of the results was done using Cell Quest software.

Induction of EAE

Groups of rats were injected in both hind footpads with a 50-μl emulsion of mineral oil containing 25 μg of guinea pig MBP and 200 μg of *M. tuberculosis* (CFA) per rat. Passive EAE was adoptively transferred by i.p. injection of 2×10^7 MBP-activated A8 clone as described (23). Clinical EAE was observed in immunized rats 10 to 12 days after guinea pig MBP/CFA induction and 4 to 5 days following administration of T cell clone. Clinical scoring was: +1, paralysis of tail; +1.5, paresis of posterior paws and ataxia; +2, paraplegia; +3, paralysis extending to thoracic spine; +4, a moribund state (23).

Measurement of IL-2 secretion

The secretion of IL-2 was measured in a bioassay using a T cell line (anti-p91-110). The line, in the rest phase, was incubated in DMEM containing 10% FCS, in 5×10^4 cells per well, with known concentrations of human rIL-2 (Cetus Corporation, Emerville, CA), and in parallel with supernatants from 24-hr culture of cell lines with irradiated thymocytes and cytokine receptor peptides. The cells were incubated at 37°C in 7% CO_2 for 48 h and thymidine incorporation was measured in the last 4 h. A standard curve was drawn from the dose response of rIL-2, and the concentrations of secreted IL-2 by the various lines were deduced from the standard curve. This assay yields results that are similar to the CTLL2 IL-2 measurement assay (24).

Reverse transcription-PCR analysis

Total cellular RNA was isolated by the single-step method using the Tri-Reagent (Molecular Research Center, Cincinnati, OH). Five micrograms of total RNA were used for the reverse transcriptase (RT) reaction. RNA was incubated with oligo$(dT)_{(12-18)}$ (200 ng) for 5 min at 65°C and left to cool to 42°C. The RT reaction contained: dNTPs (0.25 mM each), RNAsin (3 U), DTT (10 mM), sodium pyrophosphate (4 mM), avian myeloblastosis virus RT (12 U), (Promega, Madison, WI) and RT buffer. The mixture was incubated for 120 min at 42°C and then heat inactivated for 5 min at 95°C. The primers used for PCR were designed with the PRIMER software (version 0.5, Whitehead Institute for Biomedical Research, Rockville, MD) from the sequences of rat cytokines obtained from the GeneBank (Table I).

The PCR reaction contained cDNA (1 μl), primers (0.3 μg), dNTPs (75 μM each), and DNA polymerase (Red Hot DNA polymerase, Advanced Biotechnologies, Leatherhead, U.K.) 2 U/reaction. PCR reactions were performed with the Stratagene Robocycler Gradient 96, and consisted of heating to 94°C for 5 min followed by 30 cycles (denaturation 94°C for 1 min, annealing 60°C for 1 min, and extension 72°C for 1 min), followed by 10 min at 72°C. PCR products were electrophoresed in 1.7% agarose gel (FMC BioProducts, Rockland, ME), containing ethidium bromide (0.2 μg/ml).

Results

Immunogenicity of cytokine receptor peptides

To investigate whether cytokine receptor peptides are involved in T-T cell interactions, we screened IL-2R α- and β-chains, IL-2, and TNFR for a motif of Lewis rat IA molecule based on peptide-binding studies combined with molecular modeling of the MHC structure (25). The peptide motif comprised five anchor residues at relative positions P1, P3, P4, P6, and P9. Negatively charged residues were highly favored at P9, and P4 and P9 were particularly important for binding (25). The prominent features of a preference for glutamic acid at the C-terminal anchor position (P9), and serine at P3 was previously noted by others (26, 27). The peptides chosen

Table I. *Cytokine primer sequences*

Cytokine	Product Size	Sequence
IL-4	294 bp	TGCTTTCTCATATGTACCGGG
		TGAGTTCAGACCGCTGACAC
IL-6	294 bp	ATTGTATGAACAGCGATGATGC
		GAGCATTGGAAGTTGGGGTA
IL-10	329 bp	GAGTGAAGACCAGCAAAGGC
		TCGCAGCTGTATCCAGAGG
IFN-γ	405 bp	ATGAGTGCTACACGCCGCGTCTTGG
		GAGTTCATTGACTTTGTGCTGG
TNF-α	551 bp	AGGAGGCGCTCCCCAAAAAGATGGG
		GTACATGGGCTCATACCAGGGCTTG
TGF-β1	458 bp	GACCTGCTGGCAATAGCTTC
		CCTTAGTTTGGACAGGATCTGG
GAPDH	408 bp	CCCACGGCAAGTTCAACGG
		CTTTCCAGAGGGGCCATCCA

Table II. *Peptide sequences*

Peptide No.	Origin	Accession No.	Position	Sequence
1	Rat IL-2Rβ	p26896	194–213	IFLETLTPDTSYELQVRVIA
2	Rat IL-2Rβ	p26896	101–120	SVDLLSLSVVCWEEKGWRRV
3	Rat IL-2Rα	p26897	101–121	TTDTQKSTQSVYQENLAGHCR
4	Rat IL-2Rα	p26897	190–210	ASEESQGSRNSFPESEACPT
5	Rat IL-2	p17108	69–89	QATELKHLQCLENELGALQRV
7	Rat TNFR1	p22934	378–397	WKEFMRLLGLSEHEIERLEL

are shown in Table II. While peptides 3 and 4 were in accord with the motif peptides, 1, 2, 5, and 7 were only partially fitting to the motif. We chose the α- and β-chains of the IL-2R as these are known to be up-regulated upon T cell activation while the γ-chain is constitutively expressed (28).

The peptides were synthesized and injected in CFA to Lewis rats. To examine the immunogenicity of peptides, day 12 draining lymph node lymphocytes were obtained and proliferative responses to the peptides were examined.

As can be seen from Figure 1*A*, all peptides except the IL-2-derived peptide induced some T cell proliferation; peptides 2 and 7 gave the best proliferative indices. To examine whether the peptides induce Ab production, Lewis rats were bled 60 days after peptide CFA inoculation. Figure 1*B* shows that peptides 1 and 4 did not induce IgG production while 2, 3, 5, and 7 induced Abs.

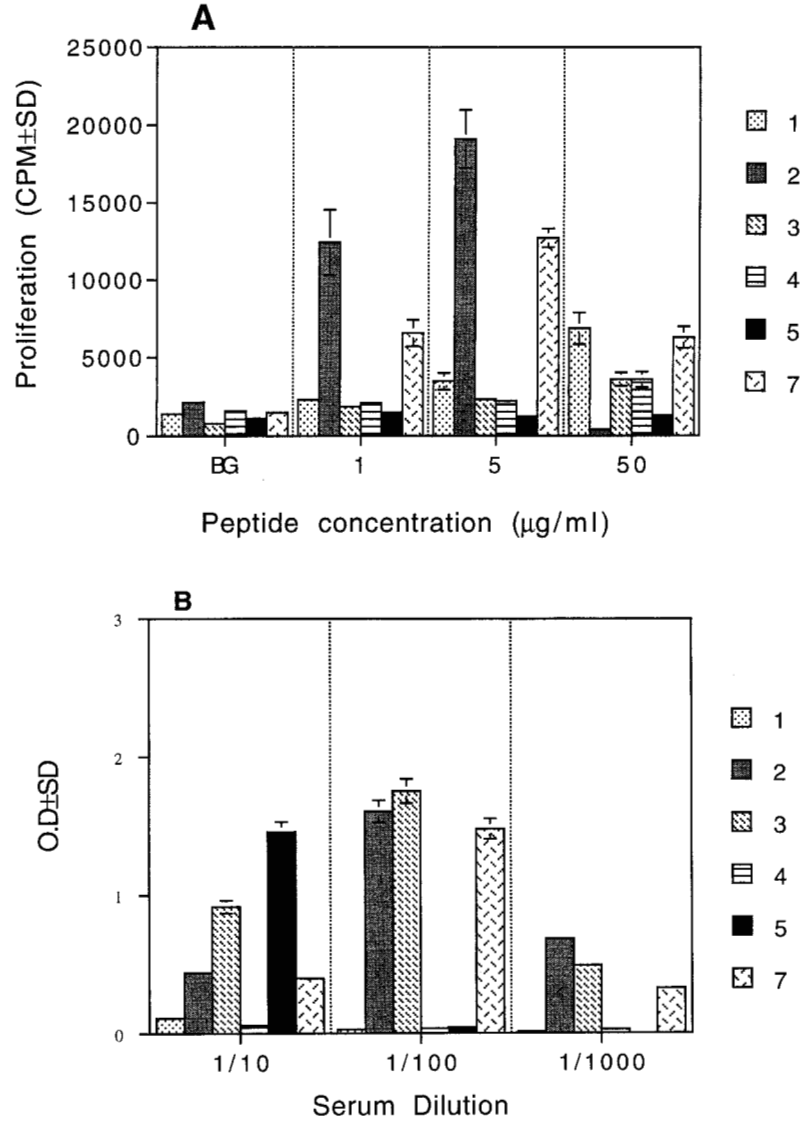

FIGURE 1. T and B cell responses to cytokine-receptor peptides in the Lewis rat. Draining popliteal lymph node cells were obtained on day 12 after peptide/CFA footpad immunization and tested in standard T cell proliferation assay. A total of 2×10^5 cells were cultured for 72 h in the presence of the indicated concentration of peptide, and the [^{3}H]thymidine incorporation was measured using MicroMate 196 Cell Harvester. The mean cpm ± SD were plotted against peptide concentration (*A*). BG denotes background, the cpm of lymphocytes in the absence of added Ag. The B cell response to cytokine-receptor peptides was examined in ELISA of sera obtained 60 days following immunization. The sera were diluted 10-, 100-, and 1000-fold. The observed OD were plotted against serum dilution (*B*).

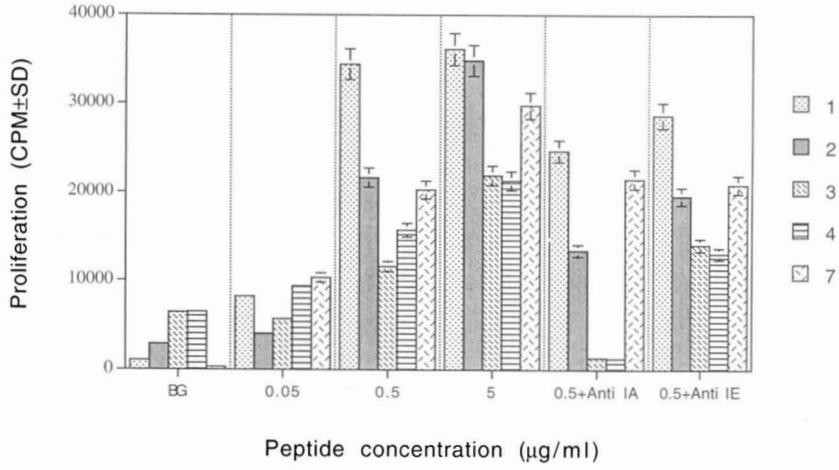

FIGURE 2. The proliferation profile of cytokine receptor-reactive T cell lines. Line cells (5×10^4) were obtained at the end of rest phase after four in vitro stimulations, and cultured in the presence of irradiated (2500 R) thymocytes (5×10^5) with the indicated concentrations of peptides. In some wells anti-IA or anti-IE mAbs were added to the wells at 10 μg/ml.

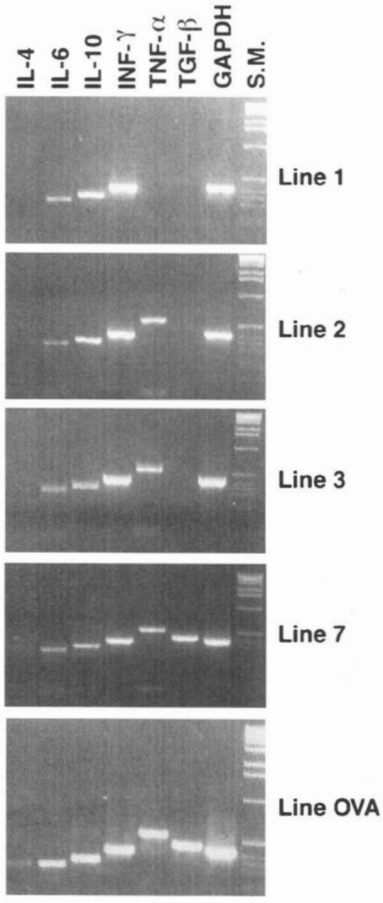

FIGURE 3. Cytokine secretion profile of anti-cytokine-reactive T cell lines. Line cells were stimulated with the specific peptide and irradiated thymocytes as APCs for 48 h. Cell blasts were isolated on Lymphoprep gradient, and total RNA was extracted with Tri-Reagent. Five micrograms of RNA were used for preparation of cDNA. PCR was performed with cytokine and GAPDH primers (see Table I). The products of PCR amplification were run on ethidium bromide-stained gel, and the results are shown in the figure.

Thus, five of six peptides chosen were able to prime T cell responses while four of six were immunogenic for B cells, leading to measurable anti-peptide IgG Abs.

Isolation of T cell lines to cytokine receptor peptides

To further delineate the in vitro and in vivo effects of cytokine-receptor reactive T cells in the Lewis rat, we generated T cell lines to peptides 1 to 4 and peptide 7. Following four in vitro stimulations with the respective peptides and irradiated thymocytes as APC, the proliferative response to various concentrations of peptide were determined. Figure 2 shows the proliferation profiles of the lines.

As can be seen from Figure 2, T cell lines were raised to peptides 1 to 4 and peptide 7. Lines 1, 2, and 7 had low background cpm (proliferation in the presence of APC only) while lines 3 and 4 had elevated background. The proliferation of lines 3 and 4 was inhibited by anti-IA mAbs while 1, 2, and 7 were not inhibited by anti-IA or IE mAbs. The elevated background of lines 3 and 4 was dependent on the addition of irradiated APC and was also inhibited by anti-IA mAb.

To further characterize the cytokine-reactive T cell lines we examined the cytokine secretion profile and the expression of Vβ genes as analyzed by FACS. IL-2 was measured using a bioassay; the secreted concentrations of IL-2 were: line 1, 44 U/ml; line 2, 30 U/ml; line 3, 12 U/ml; line 7, 10 U/ml; anti-OVA line, 1.4 U/ml, and 91-110 line, 100 U/ml. Figure 3 shows the results of expression of mRNA of IL-4, IL-6, IL-10 INF-γ, TNF-α, and TGF-β1 by RT-PCR. As can be seen, all lines expressed mRNA for IL-4, IL-6, and IL-10 INF-γ and several lines also expressed TNF-α and TGF-β. Thus, the lines were of the Th0 phenotype-expressing cytokines of both Th1 and Th2 cells (29). The results of Vβ expression by FACS (Table III) showed that all lines had a mixed pattern of Vβ composition and were not dominated by a single Vβ gene family even after eight in vitro stimulations.

Thus, unlike the T cell response to immunodominant self-Ags involved in autoimmunity that show a predominant V gene usage (30), these cells were diverse in their T cell repertoires.

Table III. *FACS analysis of Vβ gene expression of cytokine receptor lines* [3]

Line	Vβ 8.2 (%)	Vβ 8.5 (%)	Vβ 10 (%)	Vβ 16 (%)
1	1.9	4.6	6.4	9.7
2	6.0	7.5	6.5	11.0
3	14.5	2.4	1.6	16.9
4	5.8	1.5	4.1	21.8
7	3.3	3.7	7.4	6.9

[3] Line cells were examined after eight in vitro stimulations using commercial anti-Vβ mAb. The percentage of positive cells was deduced from histograms of log fluorescence intensity vs number of events, using the Cell Quest software (Becton Dickinson).

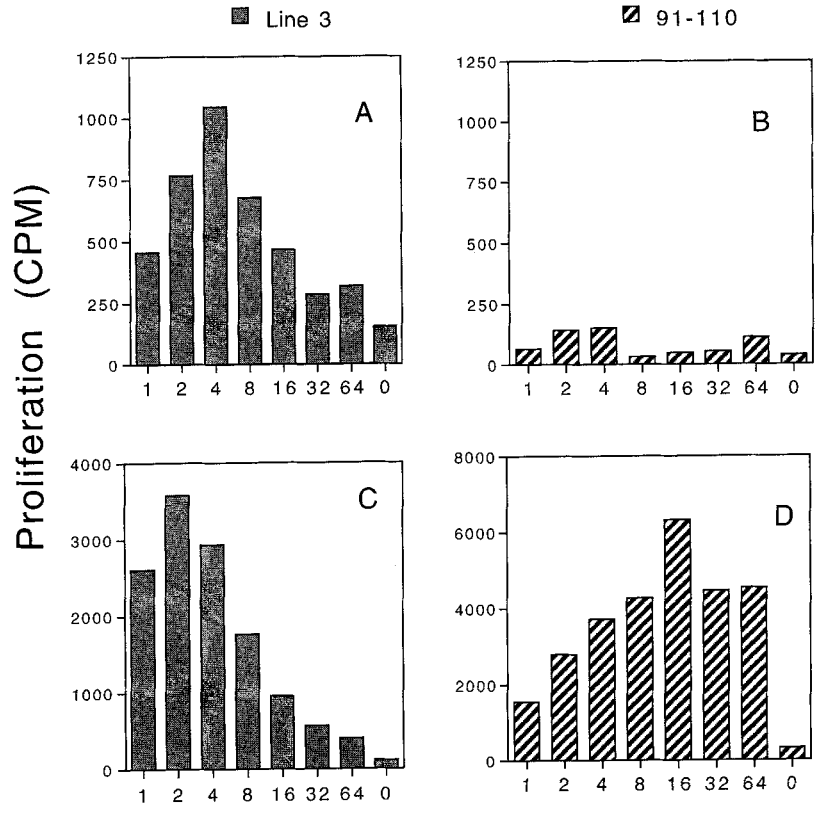

FIGURE 4. Proliferative response of cytokine receptor line 3 to A2b clone. Line 3 was cocultured with irradiated (5000 R) A2b clone in a 96-well plate; 50×10^3 line cells with decreasing concentrations of A2b clone from 50×10^3 (designated 1) in twofold dilutions (2, 4, 8, etc. indicate the reciprocal dilution of A2b). As a control line we performed the same assay with 91-110 (parts *B* and *D*). The assay was performed in the absence of peptide (*A*, no peptide 3 added and *B*, no peptide 91-110 added), and with the addition of peptide (5 μg/ml; *C*, +peptide 3, and *D* +peptide 91-110). While both lines exhibited a proliferative response when the peptide was added, only line 3 proliferated in response to the irradiated A2b clone. We tested in parallel lines 1 and 7 and these lines showed a proliferation profile similar to line 3.

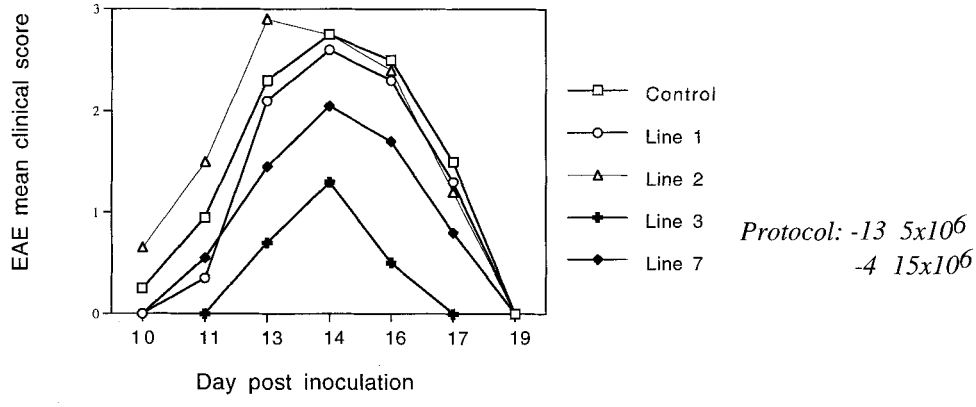

FIGURE 5. Protection against actively induced EAE by cytokine receptor lines. Groups of five rats were injected i.p. with 5×10^6 activated line cells 13 days before injection and with 15×10^6 4 days before injection of guinea pig basic protein in CFA. Rats were scored for clinical signs of EAE as indicated in *Materials and Methods*. The scores of group 3 were statistically different from the control animals when compared with the Mann-Whitney test for days 13, 14, and 16 after EAE induction (the two-tailed p value < 0.02 for each of the 3 days).

Cytokine receptor T cell lines proliferate in response to activated T clone cells

In order to examine whether these T cell lines could proliferate in response to an activated T cell clone, the lines were cocultured with activated, irradiated A2b clone in the absence of APC. As seen from Figure 4, line 3 proliferated in a dose response pattern to the irradiated clone, unlike line 91-110 that is responsive to MBP sequence 91–110 (31), thus the proliferation was not due to a secreted cytokine. Moreover, from the kinetics of secreted IL-2 we know that this cytokine is secreted on the first day of Ag-specific stimulation. In additional experiments, the same pattern of response was seen with Con A-activated lymph node cells, and there was no response to naive unstimulated lymph node cells (data not shown); thus the response to the activated T cell clone could be seen with lymph node cells, ruling out the possibility that the cytokine receptor-reactive T cells are responding to some stimulatory molecules arising in the clone after prolonged in vitro culture. In addition, the proliferative response of line 3 to irradiated A2b clone was blocked by the addition of anti-IA mAb (MRC-ox-6, 10 μg/ml) providing further evidence that the relatively mild proliferation was mediated by TCR-MHC class II interaction. A similar pattern of response to irradiated A2b was seen with lines 1 and 7 (data not shown).

In vivo function of cytokine receptor-reactive T cell lines

To test the in vivo immunoregulatory potential of cytokine receptor-reactive T cell lines, naive Lewis rats were inoculated with the various lines and the course of clone-induced EAE was examined

in the injected rats. The clinical course of EAE was not different in the line recipients as compared with the control rats; thus, the lines could not protect against clone-induced EAE (data not shown). We reasoned that it is possible that the lines were unable to exert a beneficial effect in this setting since the clone is first activated in vitro and then injected at a late stage when it is equipped with its effector functions. In actively induced EAE the animal is injected with MBP in CFA and the encephalitogenic T cell activation occurs in vivo, thus allowing the cytokine receptor T cells to affect the activation process in its induction phase. Groups of rats were injected twice with activated anticytokine receptor T line cells, and then active EAE was induced in these rats. The results of EAE clinical scores are depicted in Figure 5. The clinical course of disease was significantly suppressed in the rats receiving line 3, while in the other groups the disease was not different from the control group.

Discussion

The molecular basis of T-T interactions is not clear. Based on findings in the Ab network, including both idiotypic (7) and nonidiotypic interactions, researchers investigated similar possible interactions between T cells. The first molecule that was examined in idiotypic T-T interactions was the TCR. Using synthetic peptides from various regions of the TCR and testing for potential immunoregulatory role of T cell lines directed to these peptides several regions were found to mediate potent regulatory functions including: the CDR1 (10), CDR2 (11), CDR3 (12), and framework 3 region of the β-chain (32). Since in many of these studies the TCR peptide-specific T cell lines did not proliferate in response to the original T cell, the question raised is whether in physiologic conditions the same peptides are presented on MHC class II molecules (33). For several years it has been known that beside the TCR-based immune regulation there are T cells that proliferate in response to activated T cells irrespective of their TCR (14, 34). Since cytokine receptors are membrane proteins likely to be presented by MHC class II molecules (15, 35) and they are up-regulated during T cell activation, we designed the present study to test the possible role of cytokine receptor peptides in T-T interactions.

The results of the present study indicate that in rats, synthetic peptides derived from IL-2 and TNFR are immunogenic and induce both T and B cell responses, indicating that the lymphocytes reacting to these self-Ags were not deleted from the repertoire. Since the IL-2R is known to be expressed in the thymus (36, 37), such peptides could potentially positively select for the relevant T cells in the thymus. Indeed during studies of elution of peptides from MHC class II molecules from B cells, one of the peptides was from the IL-2R γ-chain (38). The T cell lines reactive to cytokine receptors were also stimulated by activated T cells (both lines and Con A lymph node blasts) and had a mRNA for cytokines compatible with a Th0 profile upon activation. The T cell lines were not composed of a single dominant Vβ family but were heterogenic. In testing their in vivo function, the lines could not protect against clone-induced EAE but line 3 protected rats from actively induced EAE. The mechanism of protection was not clarified, but it is possible that such cells, when confronted in vivo with activated T cells expressing MHC class II proteins (22) with cytokine receptor peptides, are activated to secrete cytokines that affect the activated T cells and prevent them from mediating the disease. Since the cytokine secretion pattern of line 3 was not different from that of the other lines, it is possible that when activated in vivo such line cells have a different cytokine secretion profile with more suppressive cytokines such as IL-10 and TGF-β1.

Our tissue culture conditions were selected for the generation of encephalitogenic Th1/Th0 type of Th cells. Since the cytokine receptor lines were selected using similar in vitro culture conditions, the lines were of the Th0 phenotype. Recent findings in the EAE model indicate that upon addition of IL-4 to the primary culture and decrease in the cell concentration it is possible to obtain Th2 type of cells (39). It is likely that cytokine receptor-reactive T cell lines that will be of the Th2 phenotype will have more potent suppressive effects on autoimmune disease.

Our underlying working hypothesis is that, upon T cell activation, cytokine receptors are up-regulated and presented on self-MHC class II molecules. These peptides are recognized by cytokine receptor-reactive T cells leading to their activation and secretion of cytokines. Depending on the nature of secreted cytokines this T-T cell interaction could lead to enhancement or suppression of the immune response generated by the original T cells. This form of immune regulation would thus recognize activated T cells by virtue of their activation without relation to their TCR. The recent demonstration that the Th cytokine profile can be modified in vitro by addition of IL-4 to the primary culture (39) may help augment the immunomodulating effects of cytokine receptor reactive T cell lines in vivo.

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