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Hierarchical signaling thresholds determine the fates of naïve T cells: partial priming leads naïve T cells to unresponsiveness

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Abstract

Differing conditions of antigen priming varying either the concentration or affinity of T cell receptor (TCR) ligands greatly alter T cell responses. Here, we demonstrate that antigen-specific $CD4^+$ naïve T cells primed with either altered peptide ligands (APLs) or a minimal concentration of antigen peptide become anergic without observable cell divisions. Transforming growth factor-β1 (TGF-β1) expression was induced 24 h following in these stimulation conditions producing anergic cells. Productively stimulated naïve T cells expressed IL-2 to differentiate into T helper 1 (Th1) cells, secreting interferon-γ (IFN-γ) upon secondary antigen stimulation; T cells primed with an APL did not secrete either interleukin-4 (IL-4) or IFN-γ, but expressed TGF-β1 and Tob, a member of the anti-proliferative gene family. Therefore, T cell responses are regulated by TCR signaling depending on the extent of TCR engagement. These results suggest that partial antigen stimulation in the periphery can induce naïve $CD4^+$T cell unresponsiveness.

Keywords: Naïve T cell; Anergy; Altered peptide ligand; TGF-β1; Tob

T cell responses are initiated by engagement of the T cell receptor (TCR) with an immunogenic peptide bound to a major histocompatibility complex (MHC) molecule. To evoke productive T cell responses, however, antigens must have either certain affinity for the TCR or high surface density, attaining an avidity surmounting the threshold of activation. The introduction of subtle amino acid changes in antigenic ligands (agonists) can induce qualitative and quantitative differences in T cell responses. An altered peptide ligand (APL) that interacts with the TCR at a lowered affinity either induces some but not all signals (partial agonism) or inhibits the activation caused by the agonist peptide (antagonism). The current kinetic model states that TCR engagement with APL/MHC is shorter in duration than that of the agonist/MHC complex due to more rapid dissociation [1], resulting in different outcomes in T cell maturation. T cells are selected in the thymus by self-peptides presented by MHC molecules. In this environment, APLs are favored to select antigen-specific T cells [2] as a stronger affinity interaction deletes T cells. Low levels of agonist peptide, however, can also positively select T cells [3]. APLs and low concentrations of antigenic peptide have also functioned similarly to alter $CD4^+$T cell polarization [4–8]. These observations suggest that the attenuation of TCR–MHC/peptide interactions by either modifying the structure of the ligand or reducing their concentration generates distinct TCR-mediated signals affecting the T cell maturation.

T cell anergy is a cellular state in which T cells fail to proliferate and secrete interleukin-2 (IL-2) upon TCR stimulation. Studies examining the molecular mechanisms of T cell anergy have used either TCR ligation in the absence of costimulatory molecules such as CD28 or stimulation using APLs in the presence of costimulation [9]. Under these conditions, while anergy results from non-productive stimulation, it is considered to be an active signaling process requiring calcium mobilization [10]. Reduced proliferation in anergic T cells likely results from high levels of p27kip1, an inhibitor of cell cycle progression [11], and Tob, a member of the anti-proliferative protein family [12]. In addition to blocking proliferation, Tob also inhibits IL-2 transcription by associating with Smad2 and Smad4 to enhance Smad binding to the -105 negative-regulatory element within

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the IL-2 promoter [12]. The GTP-bound form of Rap-1 increases in anergic T cells, which also blocks IL-2 gene expression [13]. In contrast, transforming growth factor-β1 (TGF-β1) is induced in anergic T cells upon antigen stimulation [14–16]. TGF-β1 is implicated in the induction of anergy as it blocks cell cycle progression by inducing the cell cycle regulators, p15INK4B [17] and p21cip1 [18]. The contrasting functions of IL-2 and TGF-β1 induction in T cells may regulate the nature of antigen-specific immune responses.

We demonstrate here that distinct thresholds control the induction of TGF-β1 and IL-2 in naïve $CD4^+$T cells. Non-productive priming with APLs or low peptide concentrations did not induce either cell division or IL-2 expression in naïve T cells; these conditions induced the expression of TGF-β1. Upon secondary stimulation, these anergic T cells failed to secrete IL-2, instead expressing TGF-β1 and Tob. In contrast, T cells stimulated under productive conditions differentiated into $CD4^+$T cells, secreting both IL-2 and IFN-γ in response to antigen. Therefore, the fate of naïve T cells upon antigen encounter is altered by the affinity and density of TCR ligands. Our findings suggest that antigen-primed naïve T cells that lack IL-2 expression but express TGF-β1 become unresponsive, leading to modified antigen-specific immune responses.

Materials and methods

Animals. AND transgenic mice [19], expressing the TCR specific for the C-terminal portion of cytochrome *c* peptide, obtained from Jackson laboratory (Bar Harbor, ME) were backcrossed to B10.BR for more that 10 generations and used at 8–10 weeks of age.

Peptides. Moth cytochrome *c* 88–103 (MCC) (ANERADLIAYL KQATK) and its analogs 99E (ANERADLIAYLEQATK) and 102E (ANERADLIAYLKQAEK) were synthesized and purified to >90% by Sawady Technology Ltd. (Tokyo, Japan).

T cell proliferation analysis. $CD4^+$T cells were isolated from AND splenocytes by MACS microbeads coated with anti-CD4 (Miltenyi Biotech, Bergisch Gladbach, Germany). $CD4^+$T cells (purity was >95%, 1×10^7/ml) were incubated with 1 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) in PBS for 10 minutes at 37 °C. Following three washes with RPMI 1640 medium (containing 10% FCS, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2mM L-glutamine), labeled $CD4^+$T cells (1×10^6) were cultured for 4 days in the absence or presence of peptides with irradiated antigen presenting cells (APC) (5×10^6). B10.BR splenocytes trapped in a nylon fiber column (Wako, Osaka, Japan) were used as APCs. Harvested cells were suspended in PBS containing 1% FCS and 0.01% sodium azide (PBS-Az). Following incubation with PE-conjugated anti-mouse CD4 (Pharmingen, San Diego, CA) for 30 min on ice, cells were washed in PBS-Az three times and analyzed on a FACS sorter (Becton–Dickinson, Mountain View, CA).

Primary and secondary stimulation of naïve $CD4^+$T cells. Naïve $CD4^+$T cells were isolated by serial sorting with MACS microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany). Briefly, AND splenocytes were stained with FITC-anti-CD4 (Pharmingen). $CD4^+$T cells were isolated following incubation with MACS microbeads coated with anti-FITC antibody. Anti-FITC beads were cleaved according to manufacturer's protocol. Cells were then incubated with microbeads coated with anti-CD62L. Purity of the isolated $CD4^+CD62L^+$T cells was >95%. Naïve $CD4^+$T cells (1×10^6) were then cultured with APCs (5×10^6) and mouse recombinant IL-2 (r-IL-2, 10 U/ml) in the presence or absence of peptides (MCC, 99E, or 102E). After five days, the collected $CD4^+$T cells were washed three times with medium. The cells (1×10^5) were re-stimulated with APCs (5×10^5) plus MCC peptide (1 μM) for either 24 or 48 h.

RT-PCR. Total RNA was isolated from cells with Isogen TRIzol (Wako, Osaka, Japan). cDNA was then synthesized from 5 μg total RNA using oligo(dT) primer and Super Script II (Gibco-BRL, Rockville, MD) at 42 °C for 50 min. cDNAs were amplified with each set of primers. The sequences of 5′ and 3′ primers were; IL-2: 5′-TG ATGGACCTACAGGAGCTCCTGAG-3′, 5′-GCAATATCAGAGT AACTGTTGTAAAA-3′, TGF-β1: 5′-AGATCTCCCTCGGACCTG CTGGCAGT-3′, 5′-CACGGCACTTCGGAGAGCGGGAAC-3′, HP RT: 5′-CCAGCAAGCTTGCAACCTTAACCA-3′, 5′-CAGGGGGC AACTGACTAGTAATG-3′, Tob: 5′-GGACATTGACGATGTTCG TGGCAAT-3′, 5′-TGCACTGAGGAAGAGATGGCTTTCT-3′. To amplify IL-2, TGF-β1 and HPRT cDNAs, polymerase chain reactions (PCRs) were performed for 30 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min. Thirty cycles of 94 °C for 1 min for denaturing, 60 °C for 1 min for annealing, and 72 °C for 1 min to extend served to amplify Tob cDNA. All reactions were within the linear amplification range of PCRs.

Measurement of cytokine production. All the antibodies used for ELISA (capture and detection) were purchased from PharMingen (10161D and 18172D for IL-2, 18031D and 18042D for IL-4, 18181D and 18112D for IFN-γ, 23201D and 23212D for TGF-β1). Recombinant IL-4 (19231V) and IFN-γ (19301T) were purchased from PharMingen. Recombinant IL-2 was obtained from Peprotech (Rocky Hill, NJ) and recombinant TGF-β1 was acquired from Genzyme/Techne (Minneapolis, MN). To detect TGF-β1, culture supernatants were heated at 80 °C for 10 min to convert into the active form. The lower limit of the sensitivity for IFN-γ, IL-2, and IL-4 was 160 pg/ml and that for TGF-β1 was 40 pg/ml.

Results

102E is not potent to induce proliferation in AND $CD4^+$T cells

We have previously reported that AND T cells specific for moth cytochrome $c_{88–103}$ (MCC) peptide interacting with its APLs within the thymus differentiate into T cells secreting TGF-β1 [14]. The APLs have a single amino acid substitution of K at position 99 with E (99E) or of T at position 102 with E (102E), which reduces the affinity of the ligand dramatically. These T cells secreting TGF-β1 are anergic and do not express IL-2 upon antigen stimulation. This study sought to determine if peripheral naïve T cells also give rise to similar anergic T cells as a consequence of TCR–APL/MHC interactions. First, we tested the ability of MCC and its APLs, 99E and 102E, to induce the proliferation of $CD4^+$ AND T cells. CFSE-labeled AND $CD4^+$T cells were stimulated with varying peptide concentrations (0.3–3 μM) in the presence of irradiated APCs isolated from B10.BR splenocytes (Fig. 1). Between six and seven cell divisions were measured by reductions in CFSE intensity of $CD4^+$T cells stimulated with MCC over a four-day period. The proportion of non-dividing AND T cells

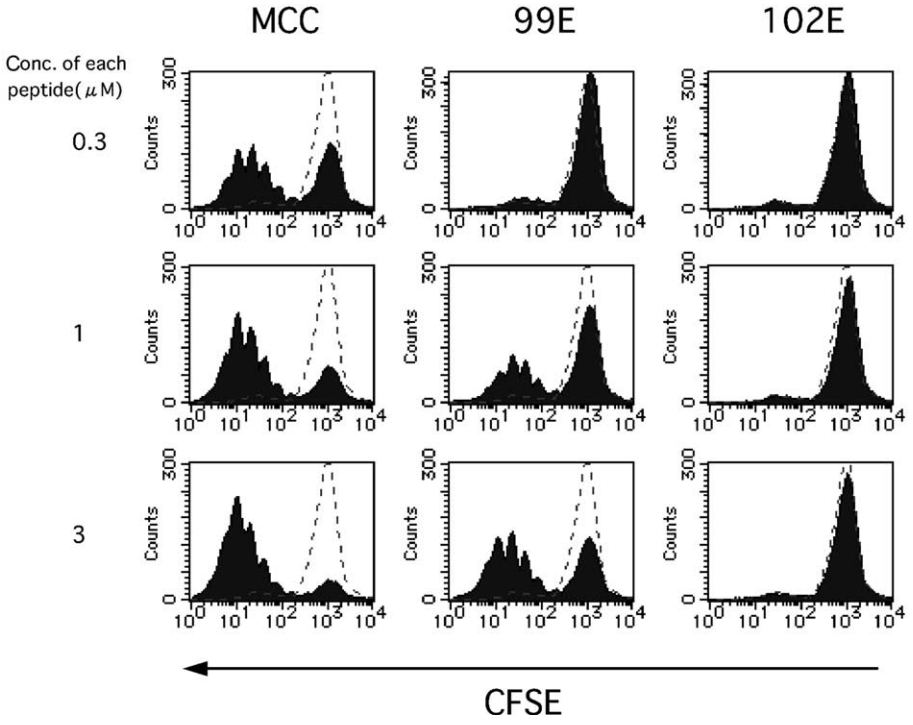

Fig. 1. Dose- and peptide-dependent priming of AND CD4$^+$T cells. CFSE-labeled CD4$^+$T cells isolated from AND mice were cultured with varying peptide concentrations in the presence of irradiated APCs. Cells collected on day 4 were stained with PE-anti-CD4. CFSE levels of gated CD4$^+$ cells, shown as filled histograms, were analyzed by FACS. The dotted histogram indicates the CFSE levels obtained from the cells cultured without peptide.

retaining the initial CFSE levels decreased with increasing concentrations of MCC peptide. Cells primed with the 99E peptide resulted in similar numbers of cell divisions, although higher concentrations of peptide were required to achieve similar proportions of AND T cells entering the cell cycle. In contrast, AND T cells stimulated with 102E peptide did not undergo any cell division. CFSE levels of cells stimulated with 102E aligned perfectly with cells cultured with APCs in the absence of peptide.

T helper 1 (Th1) differentiation is abrogated and T cells become anergic by 102E priming

We then analyzed the differentiation of AND naïve T cells primed with different doses (0.3–3 μM) of antigenic peptide and APLs. Naïve CD4$^+$T cells isolated from AND mice were cultured for five days in either the presence or absence of varying peptide concentrations. Following secondary stimulation with 1 μM MCC peptide presented by irradiated splenic APCs, we measured the production of IL-2, IL-4, and IFN-γ by ELISA. MCC induced the differentiation of CD4$^+$T cells into IFN-γ-producing cells in a dose-dependent manner. IFN-γ production by T cells primed with 3 μM 99E was similar to that induced with 0.3 μM MCC (Fig. 2A), correlating with the cell cycle progression observed during priming. IL-2, which can be induced both in naïve T cells and Th1 cells, was produced from the cells cultured without peptide and with either MCC or 99E peptide (Fig. 2B). The production of IL-2 was elevated as the concentration of the peptides increased except for the case of 3 μM MCC. Secretion of IL-4 remained below detectable levels (<160 pg/ml) in all cultures primed under different conditions (data not shown), indicating that naïve AND T cells primed with these antigens preferentially differentiated toward a Th1 phenotype. In

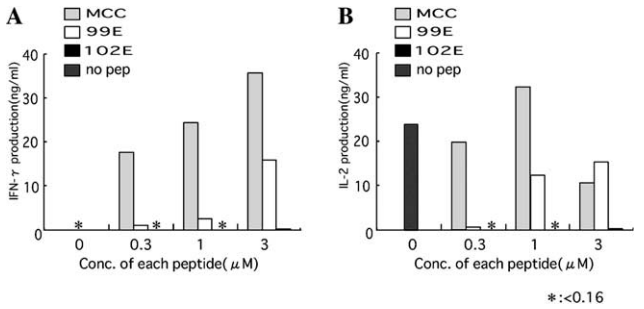

Fig. 2. Cytokine production by peptide-primed AND T cells upon secondary stimulation. Naïve CD4$^+$CD62L$^+$T cells isolated from AND mice were primed with varying peptide concentrations in the presence of 10U/ml IL-2 and APCs. Five days later, CD4$^+$T cells were re-stimulated with 1 μM MCC in the presence of irradiated APCs obtained from B10.BR mice. The concentrations of IL-2 (B) and IFN-γ (A) were determined by ELISA of cultured supernatants harvested at 24 (IL-2) and 48 (IFN-γ) hours. One representative assay of three independent experiments is shown.

contrast, neither IL-2 nor IFN-γ was detectable in cultures from $CD4^+$T cells primed with either 0.3 or 1 μM 102E peptide (Fig. 2B). Low levels of IL-2 and IFN-γ were detected only upon stimulation with 3 μM 102E. This impaired secretion of IL-2 and IFN-γ did not result from the death of AND T cells during non-productive priming, as cells cultured with APCs alone retained the ability to secrete IL-2. Therefore, AND T cells primed with either 0.3 or 1 μM 102E peptide differed from naïve T cells, likely through the receipt of signals inducing unresponsiveness.

Non-productive priming induces the expression of TGF-β1 in AND naïve T cells

We next examined the ability of the 102E peptide to induce the expression of distinct genes in AND T cells. Fig. 3 details IL-2 and TGF-β1 expression in cultured cells pulsed with 1 μM peptide. The IL-2 expression observed within 24 h of AND splenocyte stimulation with the MCC antigenic peptide disappeared after 72 h. Similar concentrations of 99E peptide induced both IL-2 and TGF-β1 within 24 hours; the expression of TGF-β1 increased in the first 48 h, remaining at high levels even after 72 h. Stimulation with 1 μM 102E, however, only induced the expression of TGF-β1. To identify if ligand density plays a role in the differential induction of cytokine expression,

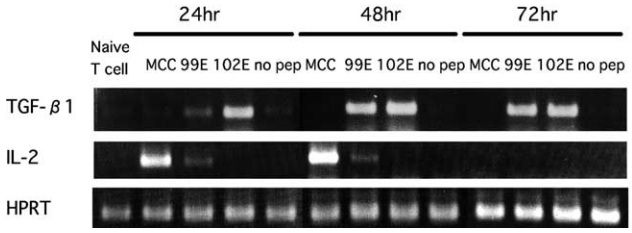

Fig. 3. Induction of IL-2 and TGF-β1 expression by MCC, 99E, and 102E peptide stimulation. AND spleen cells were cultured in the absence of presence of 1 μM peptide (MCC, 99E, or 102E). Total RNA was isolated at the specified time points, allowing the measurement of the expression of IL-2 and TGF-β1 by RT-PCR. HPRT was used as a control.

we primed AND naïve T cells with low concentrations of peptide. We measured the secretion of IL-2 and IFN-γ from the cells primed with 1×10^{-4} to 1×10^{-1} μM peptides (Figs. 4A–C). Priming with low concentrations of 99E ($1–3 \times 10^{-2}$ μM) and even lower concentrations of MCC (around 1×10^{-3} μM) rendered AND T cells unable to secrete either IL-2 or IFN-γ. Cells stimulated with 3×10^{-4} μM MCC, 3×10^{-3} μM 99E, and 3×10^{-2} μM 102E or lower concentrations of each peptide were not distinct from naïve T cells as the secretion of IL-2 upon secondary stimulation in the absence of IFN-γ production was observed. These results suggest that low densities but in certain ranges of both 99E and MCC deliver the

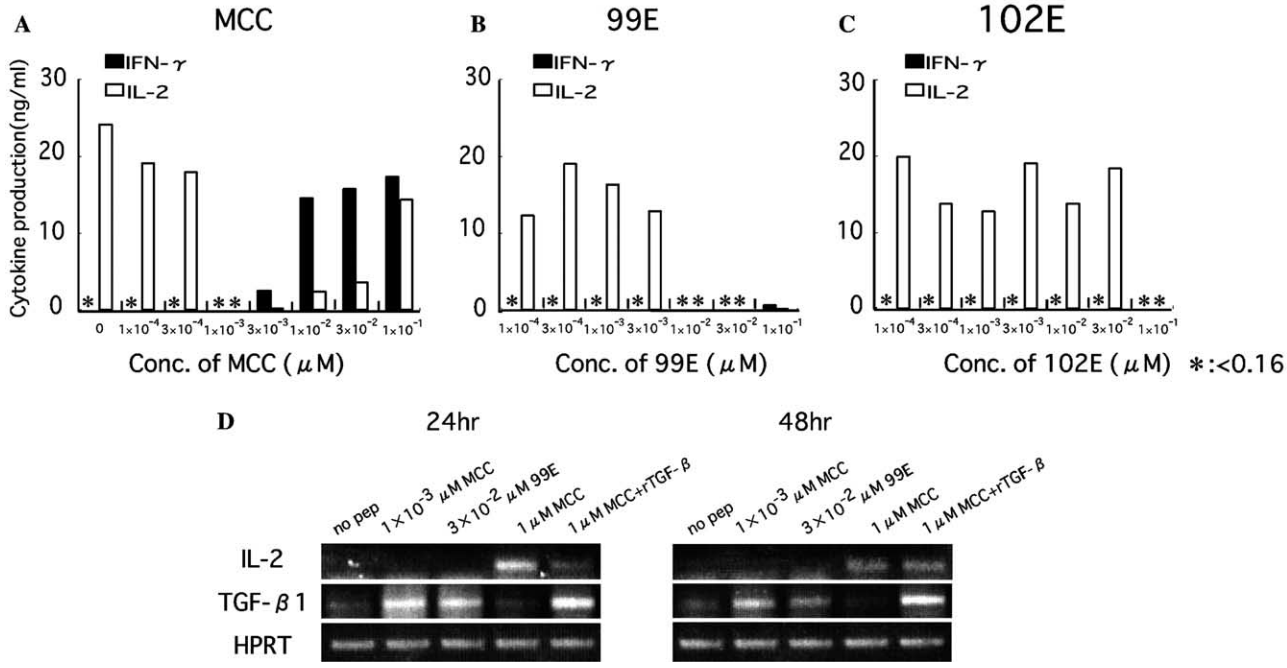

Fig. 4. Non-responsiveness induced by low doses of antigen priming. Naïve $CD4^+CD62L^+$ AND T cells were cultured as described in Fig. 2 in the presence of low doses of MCC (A), 99E (B) or 102E (C). Production of IL-2 and IFN-γ was measured by ELISA. Mean values of duplicated assays are shown. Values below the detection limit (<0.16 ng/ml) are indicated by an asterisk (*). (D) The expression of IL-2 and TGF-β1 following primary non-productive stimulation was measured by RT-PCR. Lane 1: no peptide, lane 2: 1×10^{-3} μM MCC, lane 3: 3×10^{-2} μM 99E, lane 4: 1 μM MCC, and lane 5: 1 μM MCC+10 ng/ml TGF-β1.

Table 1
Abs of neutralizing TGF-β1 cannot negate the induction of anergy by 102E

Priming conditions	Secondary conditions: $CD4^+$T cells + APC + MCC	
	IFN-γ (ng/ml)	IL-2 (ng/ml)
Naïve $CD4^+$T cells+APC+rIL-2		
+MCC	15.4 ± 7.2	23.3 ± 9.1
+MCC+rTGF-β1	<0.16	<0.16
+MCC+rTGF-β1+anti-TGF-β1	9.1 ± 2.2	18.1 ± 3.1
+102E	<0.16	<0.16
+102E+anti-TGF-β1	<0.16	<0.16

Naïve $CD4^+$T cells (1×10^6) from AND mice were cultured with APCs (5×10^6), rIL-2 (10 U/ml) and either MCC (1 μM) or 102E (1 μM) peptide. Neutralizing antibodies against TGF-β1 (1 μg/ml) and rTGF-β1 (10 ng/ml) were added in the specified cultures. $CD4^+$T cells (1×10^5), harvested 5 days later, were restimulated with APCs (5×10^5) plus MCC (1 μM). Concentrations of IL-2 and IFN-γ were determined by ELISA at 24 or 48 h, respectively. Data are presented as means ± SD.

same signals as the 102E peptide in higher concentrations. IL-2 expression was diminished in cells primed with low densities of MCC (1×10^{-4} μM) and 99E (3×10^{-2} μM), and instead replaced by significant TGF-β1 expression (Fig. 4D). Anergy, defined as the absence of IL-2 and IFN-γ secretion upon secondary stimulation, was also achieved by the addition of exogenous TGF-β1 (10 ng/ml)

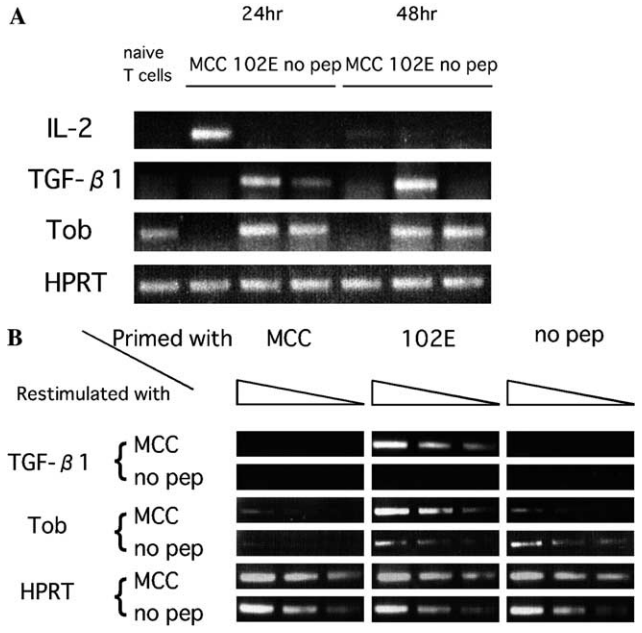

Fig. 5. Induction of expression of TGF-β1 and Tob upon secondary MCC stimulation of anergy-induced AND T cells. (A) Naïve AND $CD4^+$T cells were stimulated in the absence or presence of either 1 μM MCC or 102E with APCs. RT-PCR was performed using specific primers for IL-2, TGF-β1, and Tob. HPRT was used as a control. (B) Naïve AND T cells were cultured for 5 days as described in Fig. 2. Total RNA was prepared 24 h after secondary stimulation with 1 μM MCC plus APCs or APCs alone. RT-PCR detected the expression of TGF-β1 and Tob using 2-fold serial dilutions of isolated cDNAs.

into cultures primed with 1 μM MCC (Table 1). Elevated levels of TGF-β1 expression were observed in AND naïve T cells stimulated with 1 μM MCC in the presence of exogenous TGF-β1 (Fig. 4D). Those T cells, however, retained the expression of IL-2, indicating that the signals induced by exogenous TGF-β1 could not shut off IL-2 induction. As priming with 1 μM of 102E peptide induced TGF-β1 expression (Fig. 3), we examined the dependence of anergy induction on TGF-β1. T cells primed with 102E in the presence of neutralizing anti-TGF-β1 antibodies retained the potential to become anergic (Table 1). Therefore, while anergy induction by non-productive priming induced TGF-β1 expression, it was not mediated by the function of TGF-β1.

Anergic AND T cells induced by 102E priming express the TGF-β1 and the Tob genes upon secondary antigen re-stimulation

T cell anergy is characterized as impaired proliferative responses and the absence of IL-2 expression. Tob, an anti-proliferative mediator, plays an important role in the induction of T cell anergy. This information prompted us to analyze Tob expression in these anergic T cells. Tob was expressed in naïve T cells prior to stimulation [12] (Fig. 5A). Stimulation with 1 μM MCC diminished the expression of the Tob within 24 h. The levels of Tob expression were not suppressed in cells primed with either 1 μM 102E or in the absence of peptide. Interestingly, the expression of Tob was induced in the 102E-primed cells following secondary stimulation with MCC; this induction, however, was not significant in MCC-primed or cells primed in the absence of peptide. These 102E-primed cells also expressed TGF-β1 but not IL-2 gene following re-stimulation with 1 μM MCC. These data indicate that cytokine expression profile in anergic AND T cells is altered. In these T cells, TCR ligation induces Tob, which can function together with TGF-β1 to suppress T cell activation. Thus, AND naïve T cells either differentiated into Th1 or became anergic, depending on the affinity and density of the peptide used for primary stimulation. The expression of TGF-β1 was upregulated by non-productive priming, eventually correlating with the induction of anergy in these T cells.

Discussion

While IL-2 is a T cell growth factor that promotes T cell proliferation, one of the effects of TGF-β1 on T cells is to inhibit IL-2-dependent T cell proliferation [20]. The expression of either IL-2 or TGF-β1 is induced by different thresholds of TCR activation in $CD4^+$ antigen-specific naïve T cells. Priming of naïve T cells inducing IL-2 expression was followed by proliferation, while

priming with APLs or low concentrations of antigenic peptide instead induced the expression of TGF-β1. These results suggest that TCR engagement with low doses of antigen or APLs induces distinct signaling pathways in T cells from those induced by stimulation with higher levels of agonist peptide. Several studies have reported that antigen doses determine Th cell development [5–8]. The maturation of IL-4 producing Th2 cells was promoted by the stimulation of antigen-specific naïve T cells with either APLs or low peptide concentrations, while higher antigen doses favored the differentiation of IFN-γ producing Th1 cells [5,8]. The duration of cultures can also affect the differentiation; high level of stimulation induced both Th1 and Th2 responses in short term, whereas high doses of antigen peptides led to Th1 development preferentially over the long-term cultures [4]. Furthermore, the modulation of soluble factors in the culture by APLs and antigen doses was the driving force of the polarization to either a Th1 or Th2 phenotype [7]. We could not detect IL-4 in cultures with both low and high concentrations of peptide; the expression of IL-4, measured by RT-PCR following primary stimulation, was also very low (data not shown). It is not clear why Th2 cells did not develop in our system, however, the culture conditions we used may have preferentially supported IFN-γ producing T cells.

Differentiation toward Th1/Th2 effector T cells requires T cell activation and proliferation. Our study demonstrated that naïve T cells primed to express TGF-β1 became anergic T cells in the absence of cell division. As engagement of cytotoxic T Lymphocyte-associated antigen 4 (CTLA-4), a costimulatory molecule known as a negative regulator of T cell responses induces TGF-β expression [21], anergy induction, leading to the TGF-β1 expression may also involve inhibitory signals. The engagement of TCR by APLs induces partial phosphorylation of the TCRζ chain [22,23], which influences the activation of downstream signaling pathways by the lack of association with the ZAP-70 protein-tyrosine kinase. The introduction of a partially phosphorylated TCRζ chain into T cells inhibits T cell activation [24]. Engagement of the TCR by APLs induced anergy [25] and resulted in increased TGF-β1 expression in human Th0 clones [26]. The molecules downstream of partially phosphorylated TCRζ signaling leading to altered cytokine expression, however, remain unknown. Additional environmental factors specific to APCs may also be involved in anergy induction and Th1/Th2 differentiation. While AND T cells stimulated with 102E expressed the TGF-β1 gene in response to secondary MCC stimulation, these cells failed to secrete detectable TGF-β1 and IL-10 (data not shown), suggesting the possibility that TGF-β1 remains associated with a cellular membrane [27]. It is also conceivable that modulations in chromatin levels are required for the stable production and secretion of these cytokines [28]. In our study, TGF-β1 expression was examined in the presence of APCs that may express TGF-β1. AND T cells, however, following stimulation with anti-CD3 antibodies also expressed TGF-β1 over a relatively large range of concentrations (data not shown). Therefore, elevation of TGF-β1 expression in non-productively primed cultures is thought to be derived from the T cells. It remains to be determined which signals can induce the expression of TGF-β1 in T cells.

AND T cells primed with 1 μM 102E became anergic, exhibiting reduced secretion of IL-2 and IFN-γ. These T cells expressed TGF-β1 upon secondary MCC stimulation, similar to the phenotype observed for anergic AND T cells induced in vivo by self-antigen expression [14]. The anergic status of 102E-induced T cells was confirmed by Tob expression. Tob inhibits cell growth by suppressing cyclin D1 expression, which controls cell cycle progression [29]. Tob expression in naïve T cells was downregulated by MCC stimulation. In contrast, the upregulation of Tob expression upon secondary antigen stimulation was observed in T cells primed with 102E, indicating that antigen stimulation in these T cells transduced opposing signals that suppressed T cell activation. Anergy induction can be correlated with TGF-β1 expression that activates the Smad signaling pathway. The ability of TGF-β1 to directly induce T cells unresponsiveness remains unclear as neutralizing antibodies against TGF-β1 did not block 102E-primed anergy induction. The regulation of Tob expression has not been examined in T cells, however, TGF-β1 induced following secondary stimulation may induce Tob expression, as BMP stimulation induces Tob expression in osteoblasts [30]. Identifying the molecules responsible for anergy induction observed both in vivo and in vitro by partial engagement of the TCR will be necessary in the future to complete our understanding of how they participate in controlling the induction of immune responses and tolerance.

Acknowledgments

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