a period of months to years (*1*). Diabetic complications similarly progress over a period of several years. Hemoglobin-AGE measurements may prove useful in the evaluation of the pharmacological efficacy of newly emerging drugs aimed at inhibiting the advanced glycosylation pathway. The demonstration of decreased Hb-AGE levels as a result of aminoguanidine therapy provides the first direct evidence of the efficacy of this drug in preventing advanced glycosylation in human subjects.

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20. We are grateful to M. Yamin and K. Manogue for helpful discussions. The expert technical assistance of H. Fuh and E. Dumaguing is acknowledged. Supported by the Brookdale Foundation and NIH grant DK19655-15.

13 May 1992; accepted 4 August 1992

Negative Selection of Precursor Thymocytes Before Their Differentiation into $CD4^+CD8^+$ Cells

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Thymic selection of the developing T cell repertoire is thought to occur at the $CD4^+CD8^+$ stage of differentiation and to be determined by the specificity of the T cell receptors (TCRs) that $CD4^+CD8^+$ thymocytes express. However, TCR signals can inhibit the differentiation of precursor thymocytes into $CD4^+CD8^+$ cells, which suggests that selection might occur earlier than thought. Indeed, in a negatively selecting male thymus, $CD4^-CD8^{lo}$ precursor thymocytes that express a transgenic TCR to male antigen are developmentally arrested as a consequence of antigen encounter and fail to become $CD4^+CD8^+$. Thus, negative selection can occur before the $CD4^+CD8^+$ stage of differentiation.

T cell differentiation in the thymus consists of an ordered sequence of developmental steps that is influenced by the specificity of the TCR that individual thymocytes express (*1–4*). TCR-specific selection events are thought to act on thymocytes at the $CD4^+CD8^+$ stage of differentiation, selecting individual thymocytes for either deletion or further differentiation (*1–8*). $CD4^+CD8^+$ thymocytes appear to be the focus of thymic selection because their numbers are depleted in TCR-transgenic mice from negatively selecting mouse strains (*3, 4, 6, 7*) presumably because $CD4^+CD8^+$ transgenic thymocytes encounter antigen and are deleted. However, it is also possible that $CD4^+CD8^+$ transgenic thymocytes are low in number in negatively selecting strains because they fail to develop in these mice. To assess this latter possibility, we have examined the effect of TCR-mediated signals on the generation of $CD4^+CD8^+$ thymocytes from their immediate precursor cells in both normal mice and mice that express transgenic TCRαβ molecules (Tg-TCRαβ) specific for the antigenic complex created by the male antigen H-Y and $H\text{-}2D^b$ (D^b).

The immediate precursors of $CD4^+CD8^+$ thymocytes are $CD4^-CD8^{lo}$ thymocytes that constitute all the $CD4^-CD8^+$ cells present in the fetal thymus because mature $CD4^-CD8^+$ thymocytes do not appear until after birth (*1, 9*). To further enrich fetal thymocyte populations from both normal and Tg-TCRαβ mice for precursor cells, we eliminated thymocytes that had already become $CD4^+CD8^+$ in vivo by treatment with a monoclonal antibody (MAb) to CD4 and complement (C) (Fig. 1). Such $CD4^-$ fetal thymocyte populations consisted of two immature subsets that were distinguishable by their expression of CD8, being either $CD4^-CD8^-$ or $CD4^-CD8^+$ (Fig. 1). Placement of such $CD4^-$ fetal thymocytes into suspension culture resulted in the generation of $CD4^+CD8^+$ cells (Fig. 1), all of which were derived from $CD4^-CD8^+$ precursor cells. The derivation of these cells was determined by experiments in which precursor thymocytes were physically separated by electronic cell sorting into $CD4^-CD8^-$ and $CD4^-CD8^+$ cells (Fig. 1); $CD4^-CD8^-$ thymocytes did not undergo any detectable phenotypic change in culture (Fig. 1). Thus, precursors for $CD4^+CD8^+$ thymocytes could be uniquely identified among $CD4^-$ fetal thymocytes as $CD8^+$ cells and could be induced to further differentiate into $CD4^+CD8^+$ thymocytes by placement in suspension culture.

Studies with adult rat thymocytes have suggested that differentiation of cells expressing no CD4 and relatively little CD8

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($CD4^-CD8^{lo}$ cells) into $CD4^+CD8^+$ thymocytes can be inhibited by antibody-induced TCR cross-linking (*10*), but it has been debated whether $CD4^-CD8^{lo}$ precursor thymocytes even express the TCRs on their surface (*9–11*). However, small amounts of the TCRs were detected on $CD4^-CD8^{lo}$ murine precursor thymocytes from normal fetal mice by direct staining with fluorescein isothiocyanate (FITC)–labeled MAb to TCRβ, which was specifically inhibited by MAb to TCRβ but not by tenfold higher concentrations of MAb to CD3ε (Fig. 2A). We then examined whether antibody-induced TCR signals inhibited the differentiation of murine $CD4^-CD8^{lo}$ fetal precursor thymocytes. Suspension culture of $CD4^-CD8^{lo}$ fetal thymocytes resulted in the appearance of surface CD4 molecules; this appearance was quantitatively inhibited by plate-bound MAb to TCRβ (Fig. 2B). MAb to TCRβ was significantly more inhibitory in plate-bound form than in soluble form, which indicates a requirement for TCR cross-linking (Fig. 2B). The inhibitory effect of MAb to TCRβ was specific because MAb to TCRδ had no effect. Finally, TCR cross-linking induced by plate-bound MAb to TCRβ inhibited the differentiation of $CD4^-CD8^{lo}$ precursor thymocytes into $CD4^+CD8^+$ cells and did not simply induce a redistribution of CD4 molecules because the cross-linking also inhibited the appearance of CD4 mRNA (Fig. 2C).

To address the possibility that antigen engagement of TCR complexes might clonally regulate the differentiation of $CD4^-CD8^{lo}$ precursor cells into $CD4^+CD8^+$ thymocytes, we analyzed precursor thymocytes that expressed the TCRs with a defined antigen specificity, namely D^b+H-Y. Fetal mice that expressed the anti-male TCRαβ transgene were obtained from normal C57BL/6 (*H-2*^{b}) pregnant females (B6) that had been impregnated by a homozygous Tg-TCRαβ male and so were themselves heterozygous for the TCRαβ transgene. Approximately 50% of $CD4^-CD8^{lo}$ cells from *H-2*^{b} Tg-TCRαβ mice became $CD4^+$ during 12-hour suspension cultures, and their differentiation into $CD4^+CD8^+$ thymocytes was blocked by MAbs that bind and cross-link the transgenic TCR (MAbs to TCRβ and to TCR-V_β8) (Fig. 3). The differentiation of Tg-TCRαβ precursor cells was also inhibited by antigen-presenting cells (APCs) from male *H-2*^{b} nude mice that express the antigenic ligand (D^b+H-Y) for the TCR transgene but not by APCs from female *H-2*^{b} nude mice (Fig. 3). Thus, antigen-induced developmental arrest can occur before the $CD4^+CD8^+$ stage of development.

If antigen-induced developmental arrest of $CD4^-CD8^{lo}$ Tg-TCRαβ thymocytes occurred in vivo, $CD4^-CD8^{lo}$ precursor thymocytes from negatively selecting Tg-TCRαβ male mice should be arrested as a consequence of having encountered the male antigen in vivo. Consistent with this prediction, a significant fraction of pooled

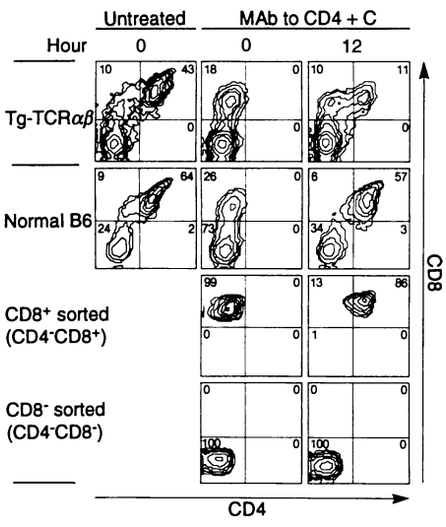

Fig. 1. Fetal $CD4^-CD8^+$ thymocytes become $CD4^+CD8^+$ cells in suspension culture (*14*). Thymocytes were obtained from normal C57BL/6 (B6; *H-2*^{b}) or Tg-TCRαβ mice on day 19 of gestation. Where indicated, thymocytes were further treated with MAb to CD4 and C to remove cells that had already become $CD4^+CD8^+$ cells in vivo. The indicated cell populations were then placed in single-cell suspension culture for 12 hours. Cell populations were analyzed by two-color flow cytometry for CD4/CD8 expression. As indicated in the lower panels, day 19 fetal thymocytes treated with MAb to CD4 and C were also electronically sorted into $CD8^+$ and $CD8^-$ cell populations and then cultured in single-cell suspension for 12 hours. Numbers in each box of contour diagrams indicate the frequency of cells in that box. After the 12-hour culture, $CD4^+CD8^+$ cells were evident in all groups except those from $CD4^-CD8^-$ cells.

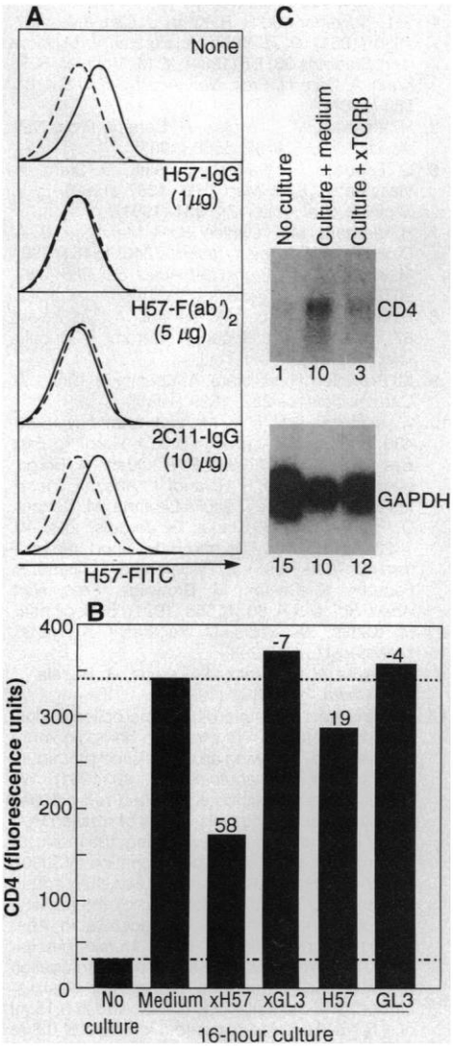

Fig. 2. TCRs on $CD4^-CD8^+$ precursor thymocytes from normal fetal mice could generate signals that inhibit differentiation into $CD4^+CD8^+$ cells. (**A**) Specificity of TCRβ staining of fetal $CD4^-CD8^+$ thymocytes from normal mice. $CD4^-$ day 19 fetal B6 thymocytes were analyzed for TCRβ and CD8 staining by two-color flow cytometry (*14*). Single-color histograms of TCRβ staining are displayed on electronically gated $CD8^+$ cells. TCR expression was determined by staining with FITC-labeled MAb to TCRβ (H57). The ability of intact or $F(ab')_2$ fragments (*15*) of unlabeled MAbs to block staining by MAb to TCRβ was determined by incubating cells with blocking antibody or $F(ab')_2$ fragments for 30 min at 4°C before staining with either MAb to TCRβ (solid lines) or control MAb (dashed lines). (**B**) Effects of TCR signals on surface CD4 expression by normal $CD4^-CD8^+$ precursor thymocytes in suspension culture. $CD4^-$ thymocytes from day 19 fetal B6 mice were cultured for 16 hours with MAbs to TCR and assessed for CD4 and CD8 expression by two-color flow cytometry (*14*). MAbs to TCRβ and TCRδ were H57-597 (*15*) and GL3 (*16*), respectively. Plate-bound MAbs (×H57 and ×GL3) were immobilized at 50 μg/ml, and soluble MAbs (H57 and GL3) were added at 50 μg/ml. CD4 fluorescence intensity on electronically gated $CD8^+$ cells was quantitated in linear fluorescence units (*17*). Percent inhibition of CD4 fluorescence relative to groups cultured in medium alone was also calculated for each experimental group (*17*) and is shown above each bar. (**C**) Effects of TCR cross-linking on steady-state mRNA in cultured $CD4^-$ fetal thymocytes from normal mice. $CD4^-$ thymocytes from day 19 fetal B6 mice were cultured for 6 hours in the absence or presence of plate-bound MAb to TCRβ (×TCRβ). Total cellular RNA (5 μg) from the indicated cells were analyzed by Northern (RNA) blot hybridization with the indicated probes (*18*). Numbers under each lane indicate relative intensities of bands as determined by densitometry. GAPDH is a housekeeping enzyme that was used as an internal standard for the relative amounts of RNA loaded in each lane.

$CD4^-CD8^{lo}$ fetal thymocytes from unsexed *H-2*^{b} Tg-TCRαβ fetal mice did not differentiate in suspension culture into $CD4^+CD8^+$ cells, whereas essentially all $CD4^-CD8^{lo}$ precursor thymocytes from normal *H-2*^{b} mice did differentiate into $CD4^+CD8^+$ cells (Figs. 1 and 3). Consequently, we separately assessed the ability of $CD4^-CD8^{lo}$ precursor thymocytes from male and female *H-2*^{b} transgenic fetuses to differentiate into $CD4^+CD8^+$ cells in vitro (Fig. 4A). Virtually all precursor thymocytes from *H-2*^{b} female transgenic mice and unsexed *H-2*^{d} transgenic mice (neither of which expressed the D^b+H-Y antigenic ligand) became $CD4^+CD8^+$ in culture (Fig. 4A). In contrast, most precursor thymocytes from *H-2*^{b} male transgenic mice (which expressed the D^b+H-Y antigenic ligand) did not become $CD4^+CD8^+$ in culture (Fig. 4A), which indicates that they had lost their ability to differentiate in vitro into $CD4^+CD8^+$ cells. The few precursor thymocytes from male transgenic mice whose developmental capacity had not been arrested may represent cells that had not yet encountered antigen.

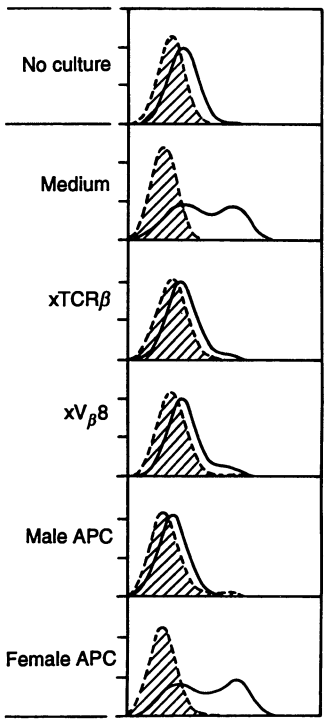

Fig. 3. Generation of $CD4^+CD8^+$ cells from Tg-TCRαβ fetal $CD4^-CD8^+$ thymocytes in culture is inhibited by TCR cross-linking and by antigen. $CD4^-$ thymocytes from day 19 *H-2*^{b} Tg-TCRαβ fetal mice were cultured for 12 hours in medium or in the presence of plate-bound MAbs or APCs as indicated (*19*). Fluorescence profiles of $CD8^+$-gated cells stained with MAb to CD4 (open solid lines) and control MAb (shaded dashed lines) are shown. At the end of culture, cell recoveries were >90% in all groups.

To confirm that in vivo exposure to the male antigen affected Tg-TCRαβ thymocytes at the $CD4^-CD8^{lo}$ precursor stage of differentiation, we examined CD5 expression on precursor thymocytes from normal and transgenic fetal mice because CD5 expression increases as thymocytes differentiate and undergo thymic selection events (*1*). All $CD4^-CD8^{lo}$ fetal thymocytes from normal mice were $CD5^{lo}$ but increased their expression of CD5 to intermediate levels ($CD5^{int}$) after activation by phorbol 12-myristate 13-acetate (PMA), which activates protein kinase C and mimics many TCR signals (Fig. 4B). $CD4^-CD8^{lo}$ fetal thymocytes from pools of unsexed *H-2*^{d} Tg-TCRαβ fetal mice were similarly $CD5^{lo}$ (Fig. 4B). In contrast, $CD4^-CD8^{lo}$ fetal thymocytes from pools of unsexed *H-2*^{b} Tg-TCRαβ fetal mice contained a subpopulation of $CD5^{int}$ cells in the absence of any intentional stimulation (Fig. 4B). $CD4^-CD8^{lo}$ fetal thymocytes from *H-2*^{b} Tg-TCRαβ male and female mice differed in their CD5 expression, with most Tg-TCRαβ male mice being $CD5^{int}$, which indicates that they have already encountered antigen in vivo (Fig. 4B). Although they were $CD5^{int}$, the $CD4^-CD8^{lo}$ thymocytes from Tg-TCRαβ male mice were not $CD4^-CD8^+$ mature cells because they remained phenotypically immature cells by other developmental markers; they were heat-stable antigen^{hi} and intercellular adhesion molecule (ICAM)-1^{hi} (*12*). Thus, $CD4^-CD8^{lo}$ precursor thymocytes in Tg-TCRαβ male mice encounter the male antigen in the thymus and, as a result, are unable to further differentiate into $CD4^+CD8^+$ cells.

Inhibition by antigen of the differentiation of $CD4^-CD8^{lo}$ precursor cells into $CD4^+CD8^+$ thymocytes is a mechanism of negative selection that occurs earlier than other described selection events that act on $CD4^+CD8^+$ thymocytes (*2–8*) or more mature cells (*13*). Because most $CD4^-CD8^{lo}$ precursor thymocytes from negatively selecting mice could not differentiate into $CD4^+CD8^+$ cells, antigen-induced developmental blockade of precursor thymocytes is probably the main cause of the low number of $CD4^+CD8^+$ thymocytes in negatively selecting *H-2*^{b} Tg-TCRαβ male mice. However, the concept of developmental blockade of precursor thymocytes is complementary to, and not mutually exclusive of, the concept of subsequent antigen-induced deletion of $CD4^+CD8^+$ thymocytes in these animals. Antigen-induced TCR signals can be generated only in precursor thymocytes with TCRs that engage antigen in the absence of CD4 and in the presence of little CD8. Thus, antigen-induced developmental blockade of precursor thymocytes might be expected to primarily affect thymocytes that express high-affinity TCRs with major histocompatibility complex (MHC) class I restriction specificities. In fact, negatively selecting thymuses of transgenic mice that express different TCR transgenes restricted by MHC class I determinants are all depleted of early $TCR^{lo}CD4^+CD8^+$ cells (*3, 4, 6, 7*), consistent with antigen-induced developmental arrest of precursor thymocytes being the primary mechanism of negative selection in these experimental animals.

The ultimate fate of antigen-stimulated $CD4^-CD8^{lo}$ precursor thymocytes is unclear. These thymocytes are probably not long-lived end-stage cells because they do not accumulate in negatively selecting mice. However, they do not appear to

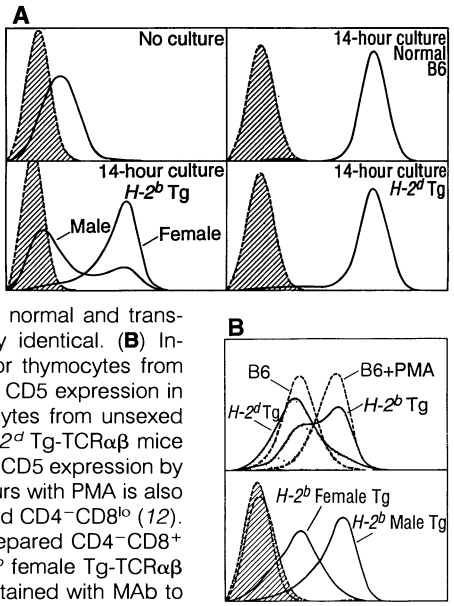

Fig. 4. Most fetal precursor thymocytes from *H-2*^{b} Tg-TCRαβ male mice cannot become $CD4^+CD8^+$. (**A**) The inability of fetal $CD4^-CD8^+$ thymocytes from *H-2*^{b} male Tg-TCRαβ mice to generate $CD4^+CD8^+$ cells in culture. $CD4^-$ day 19 fetal thymocytes from normal B6 mice, sexed *H-2*^{b} Tg-TCRαβ mice (*20*), and unsexed *H-2*^{d} Tg-TCRαβ mice were cultured for 14 hours. Fluorescence profiles of $CD8^+$-gated cells stained with MAb to CD4 (open solid lines) and control MAb (shaded dashed lines) were obtained before culture and after 14 hours of culture. Fluorescence profiles of normal and transgenic thymocytes before culture were essentially identical. (**B**) Increased CD5 expression on $CD4^-CD8^{lo}$ precursor thymocytes from *H-2*^{b} male Tg-TCRαβ mice (*21*). Top panel shows CD5 expression in freshly prepared $CD4^-CD8^+$ day 19 fetal thymocytes from unsexed *H-2*^{b} Tg-TCRαβ mice (shaded curve), unsexed *H-2*^{d} Tg-TCRαβ mice (stippled curve), or normal B6 mice (dashed lines). CD5 expression by $CD4^-CD8^{lo}$ fetal B6 thymocytes cultured for 12 hours with PMA is also shown (dashed line). PMA-stimulated cells remained $CD4^-CD8^{lo}$ (*12*). Bottom panel shows CD5 expression in freshly prepared $CD4^-CD8^+$ day 19 fetal thymocytes from *H-2*^{b} male and *H-2*^{b} female Tg-TCRαβ mice. Fluorescence profiles of $CD8^+$-gated cells stained with MAb to CD5 (open solid lines) and control MAb (shaded dashed lines) are shown.

undergo programmed cell death because they do not fragment their DNA (*12*). Rather, antigen-stimulated $CD4^-CD8^{lo}$ precursor thymocytes may become $CD4^-CD8^-TCR\alpha\beta^+$ thymocytes, which would explain the disproportionate appearance of such thymocytes in Tg-TCRαβ male mice (*3*, *4*).

Our data show that TCR signals generated in $CD4^-CD8^{lo}$ precursor thymocytes can clonally regulate their differentiation into $CD4^+CD8^+$ cells. Consequently, the TCR repertoire expressed by early $CD4^+CD8^+$ thymocytes may already reflect the influence of intrathymic selection pressures.

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14. Tg-TCRαβ mice express the anti-male TCR and were originally developed by von Boehmer and colleagues (*4*). The *H-2*^{b} and *H-2*^{d} Tg-TCRαβ fetuses were obtained from C57BL/6 and BALB/c females, respectively, impregnated by a homozygous Tg-TCRαβ male mouse. Thymocytes from day 19 fetal mice were treated with RL172 MAb to CD4 [R. Ceredig, J. W. Lowenthal, M. Nabholz, H. R. MacDonald, *Nature* **314**, 98 (1985)] plus C and then separated by lympholyte-M (Cederlane Laboratories, Hornby, Ontario, Canada) centrifugation. These $CD4^-$ cells were further stained with MAb to CD8 (53-6-72; Becton-Dickinson, San Jose, CA) and sorted into $CD8^+$ ($CD4^-CD8^+$) and $CD8^-$ ($CD4^-CD8^-$) cells by a FACStar Plus cell sorter (Becton-Dickinson). Cells were cultured at 0.5 × 10^6 to 2 × 10^6 cells per milliliter in 24-well Costar plates in RPMI-1640 medium supplemented with 10% fetal bovine serum and 5 × 10^{-5} M 2-mercaptoethanol at 37°C in a humidified atmosphere with 5% CO_2. Cultured cells were stained with MAb to CD4 conjugated to FITC (Rm4-5; Pharmingen, San Diego, CA) and MAb to CD8 (53-6-72) conjugated to biotin and then with Texas red linked to avidin. The two MAbs to CD4, RL172 and Rm4-5, bind to different CD4 epitopes and do not compete with one another. All staining was performed in the presence of unlabeled MAb to the Fc receptor to block Fc receptor–mediated binding.
15. MAb to the TCRβ (H57-597) [R. T. Kubo, W. Born, J. Kappler, P. Marrack, M. Pigeon, *J. Immunol.* **142**, 2736 (1989)] was digested with immobilized pepsin (Pierce, Rockford, IL), and the purity of the resulting $F(ab')_2$ fragment was ascertained by SDS–polyacrylamide gel electrophoresis. Immunoglobulin G (IgG) fraction of MAb to CD3ε (145-2C11) [O. Leo, M. Foo, D. H. Sachs, L. E. Samelson, J. A. Bluestone, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1374 (1987)] was also used (2C11-IgG).
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17. Fluorescence units (FU) are median intensity of CD4 staining minus median intensity of control MAb Leu4 staining. We calculated median intensity by converting median log channel number to linear units with an empirically derived calibration curve for each logarithmic amplifier used. Percent inhibition of CD4 FU was calculated as 100 × (FU of cultured cells with medium alone − FU of experimental group)/(FU of cultured cells with medium alone − FU of uncultured precursor cells).
18. Total cellular RNA was prepared from the indicated cell populations, and equal amounts of RNA (5 μg) were denatured, separated by electrophoresis on agarose gels, and transferred to nylon membranes as described [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1982)]. Blots were hybridized with a 2.1-kb Eco RI fragment of CD4 cDNA [D. R. Littman and S. N. Gettner, *Nature* **325**, 453 (1987)] or 1.3-kb Pst I fragment of rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA [P. Fort *et al.*, *Nucleic Acids Res.* **13**, 1431 (1985)] that had been labeled with ^{32}P by the random primer method.
19. $CD4^-$ day 19 fetal thymocytes from pools of unsexed *H-2*^{b} Tg-TCRαβ mice were prepared, cultured, and stained (*14*). MAb with indicated specificity [50 μg/ml; either H57-597 (H57) for MAb to TCRβ or MR5-2 (Pharmingen) for MAb to $V_\beta 8$] was coated onto the culture wells. Plastic-adherent cells (APCs) were prepared from spleen cells of T cell–deficient B10-*nu/nu* mice (*5*). Where indicated, thymocytes (0.5 × 10^6 cells per milliliter) were cocultured with APCs (0.4 × 10^6 cells per milliliter) in 24-well Costar plates.
20. Fetal Tg-TCRαβ mice on day 19 of gestation were sexed according to their internal genital organs [K. Theiler, *The House Mouse: Atlas of Embryonic Development* (Springer-Verlag, New York, 1989)].
21. $CD4^-$ day 19 fetal thymocytes were stained with FITC-conjugated MAb to CD5 (53-7-3; Becton-Dickinson) and biotinylated MAb to CD8 and then TR-avidin was added. CD5 expression was analyzed on electronically $CD8^+$ gated cells. Where indicated, $CD4^-$ day 19 fetal B6 thymocytes were cultured for 12 hours with PMA (100 ng/ml; Sigma).

22. We thank H. von Boehmer and M. Steinmetz for making their TCR transgenic mouse strain available to the scientific community; B. J. Fowlkes for providing a homozygous transgenic male breeder mouse; M. Sheard for assistance in flow cytometry and cell sorting; M. Kuehn and L. Lowe for helping in the sex diagnosis of fetal mice; and J. A. Berzofsky, R. J. Hodes, and D. S. Singer for critically reading the manuscript.

5 June 1992; accepted 20 August 1992

Flow-Dependent Cytosolic Acidification of Vascular Endothelial Cells

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Hemodynamic shear stress affects endothelial cell structure and function, but little is known about the signal transduction mechanisms involved in these processes. The effect of laminar shear stress on cytosolic pH (pH_i) was examined in rat aortic endothelial cells cultured in glass capillary tubes. Shear stress forces led to a rapid decrease in pH_i (maximal effect 0.09 pH unit at 13.4 dynes per square centimeter). Removal of specific ions or addition of exchange inhibitors suggests that in vascular endothelial cells shear stress forces activate both an alkali extruder, sodium ion–independent chloride-bicarbonate ion exchange, and an acid extruder, sodium-hydrogen ion exchange; the net effect in physiologic buffer with the bicarbonate ion is a decrease in pH_i.

Shear stress forces modulate several vascular endothelial cell functions (*1*), but the signal transduction mechanisms involved in these processes have not been fully elucidated. Because shear stress leads to enhanced phosphoinositide turnover (*2*) and membrane hyperpolarization (*3*), both of which may influence cytosolic pH (pH_i) regulation, pH_i could provide a signal transduction mechanism in response to changes in flow. In addition, shear stress–induced changes in cytoskeletal organization or in agonist availability to the cell membrane might affect pH_i (*4*). To test the hypothesis that shear stress forces affect endothelial pH_i, we examined the effect of laminar flow on endothelial cells from the rat aorta cultured in glass capillary tubes (*5*).

When endothelial cells in glass capillaries were exposed to a HCO_3^--buffered solution equilibrated with 5% CO_2, the increase in laminar shear stress forces (6) caused a rapid and reversible decrease in pH_i (*7*) (Fig. 1). The threshold for this effect was below 0.5 dyne cm^{-2}, and it saturated between 6.7 and 13.4 dyne cm^{-2} (Fig. 1A). In a HCO_3^- buffer, exposure to 13.4 dyne cm^{-2} for 2 to 5 min resulted in an intracellular acidification of 0.09 ± 0.01 pH units ($n = 79$; $P < 0.0001$). A similar

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