Brief Definitive Report

Thiol-mediated Redox Regulation of Intestinal Lamina Propria T Lymphocytes

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Abstract

Intestinal lamina propria T lymphocytes (LP-Ts) have a markedly low proliferative potential both in vivo and in vitro. Here, we have identified that the capacity of antigen-presenting cells to release cysteine upon receptor–ligand interactions represents a critical parameter for proliferation of LP-Ts. The availability of cysteine is limiting for the intracellular production of glutathione, which in turn is essential for cell cycle progression. When cysteine is provided either directly or by addition of the reducing agent 2-mercaptoethanol to cystine-containing culture medium, proliferation of LP-T is fully restored. Importantly, coculture with peripheral blood monocytes that easily take up cystine, reduce cystine, and secrete cysteine also restores reactivity of LP-Ts to T cell receptor/CD3 stimulation. In marked contrast, lamina propria macrophages lack this capacity to elaborate cysteine, and thereby secure physiological unresponsiveness to antigen exposure in the intestinal microenvironment. The well-documented local recruitment of blood monocytes in inflammatory bowel disease (IBD) may thus represent an important parameter underlying hyperresponsiveness of T cells, an essential component of the pathogenesis of IBD.

Key words: macrophage • monocyte • inflammatory bowel disease • cysteine • glutathione

Introduction

Human intestinal lamina propria T lymphocytes (LP-Ts) do usually not develop systemic immune responses despite a permanent exposure to foreign bacterial and nutritional antigens. When compared with peripheral blood T lymphocytes, the reactivity of LP-Ts to in vitro stimulation by mitogenic CD3 antibodies is reduced (1), whereas experimental activation through the costimulatory receptors CD2 or CD28 is similar (1, 2). Mechanisms maintaining this "altered" state of responsiveness in the normal gut as well as the increased reactivity of LP-Ts to TCR/CD3 engagement in chronic inflammatory bowel disease (IBD) are unclear. Several hypotheses have been put forward, such as continuous antigenic stimulation that would lock LP-Ts in a hyporesponsive state (3), local production of antiinflammatory IL-10 (4), and impaired costimulation by lamina propria macrophages (LP-MOs [5]). In addition, low molecular weight nonprotein mediators with oxidative capacities produced in the gut mucosa have been proposed to be involved in descreased antigen-mediated stimulation of LP-Ts (6). Therefore, the balance of naturally occurring redoxactive substances in the microenvironment might regulate the reactivity of LP-Ts.

In this study, we provide evidence for a dominant role of thiol-mediated redoxregulation for intestinal T lymphocyte reactivity. Cysteine is the limiting substrate for the synthesis of glutathione, which is a prerequisite for cell cycle progression (7). Cysteine, but not equimolar amounts of cystine, and 2-ME (which acts by improving cellular uptake of cysteine [8]) fully restore CD3 reactivity of LP-Ts. Peripheral blood monocytes (PB-MOs) support the proliferation of T cells by release of cysteine that is triggered by CD2–CD58 interactions, whereas in contrast, LP-MOs lack this ability. Because PB-MOs infiltrate the inflamed gut in IBD, cysteine release by PB-MOs evolves as a novel mechanism of lymphocyte stimulation likely to be relevant to the pathogenesis of IBD.

Materials and Methods

mAbs and Reagents. The following mouse mAbs were affinity purified from tissue culture supernatant on protein A sepharose (Amersham Pharmacia Biotech): M1 (anti-CD2; IgG1), M2 (anti-

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CD2; IgG1), 3PT (anti-CD2; IgG2a), OKT3 (anti-CD3; IgG2a), 1A3 (anti-CD58; IgG2a), GP89 (anti-CD54; IgG2a). OKT3 (1 μg/ml) was immobilized on immunobeads (10^8/ml) coated with goat anti–mouse Ig (Irvine Scientific) for 2 h at 37°C in PBS/0.5% BSA. Recombinant human IL-2 was a gift from Biotest. 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) was purchased from Bristol Laboratories and dissolved in ethanol. Buthionine-*S*,*R*,-sulfoximine (BSO), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), LPS from *Escherichia coli* (serotype 055:B5), 2-ME, dithiothreitol (DTT), glutathione disulfide (GSSG), and hydrogen peroxide were obtained from Sigma-Aldrich, and cystine was obtained from GIBCO BRL. Dissolved cysteine (Serva) and reduced glutathione (GSH; Serva) were adjusted to pH 7.3 before use.

Preparation of Cells from Intestinal Lamina Propria and Peripheral Blood. Normal colon mucosa was obtained from patients undergoing resection for colon cancer. LP-Ts were isolated according to a modified method of Bull and Bookman (9) as described earlier (1), with the exception that the mucus was scraped off with a scalpel instead of incubating the tissue with the antioxidant DTT. In brief, the tissue was washed in HBSS without Ca^{2+} or Mg^{2+} and cut into 2–4-mm pieces, and epithelial cells were removed by incubation with EDTA. After enzymatic digestion (collagenase, deoxyribonuclease I), viable mononuclear cells were obtained by Percoll and subsequent Ficoll-Hypaque density gradient centrifugation. Cells were resuspended in a 1:1 mixture of RPMI 1640/2% FCS and autologous serum, and were allowed to adhere to plastic Petri dishes for 3 h at 37°C. Adherent cells were harvested using a rubber policeman, and were used as LP-MOs. LP-Ts were purified from nonadherent cells by E-rosette formation with SRBC (ICN Biomedicals [6]), and were 90% positive for CD3. PBMCs from the same patient were isolated by Ficoll-Hypaque density gradient centrifugation. Cells adherent to plastic Petri dishes were used as PB-MOs. All cells were finally resuspended in RPMI 1640 plus 10% FCS, penicillin/streptomycin, and 2% glutamine. In some experiments, cystine-deficient RPMI 1640 was used (Serva/BioWhittaker).

Proliferation Assay. LP-Ts (5×10^4/well) were cultured in 96-well round-bottomed microtiter plates (Costar) at 37°C and 7% CO_2. Cells were stimulated via CD3 using OKT3-coated beads (ratio beads/cells = 4:1), or via CD2 using the mitogenic mAb M1+M2 (1 μg/ml each) plus 3PT (0.33 μg/ml). PB-MOs and LP-MOs were irradiated (50 Gy) before use, and were added to LP-Ts at 30% of total cell number. Wells were pulsed with 1 μCi [^{3}H]-TdR (Amersham Pharmacia Biotech) at day 4 for 16 h, and then were harvested on glass fiber filters using an automatic cell harvester (FilterMate; Packard Instrument Co.). [^{3}H]-TdR uptake was measured in a microplate scintillation counter (TopCount; Packard Instrument Co.). Results are expressed as mean cpm of triplicate cultures ± SD.

Determination of Acid-soluble Thiol. Cysteine was determined as acid-soluble thiol essentially as described earlier (10). The cell-free supernatant (600 μl) was mixed with 150 μl EDTA (80 mM) and 150 μl TCA (30%) to precipitate protein. After incubation for 15 min on ice and centrifugation, the supernatant (267 μl) was mixed with 400 μl phosphate buffer (0.5 M; pH 7.0) and 100 μl NaOH (1 N). Finally, 33 μl DTNB (10 mM) was added, and increase of the absorption was measured at 412 nm using cysteine as a standard.

Results

Influence of Oxidants and Antioxidants on DNA Synthesis of LP-Ts. Micromolar concentrations of hydrogen peroxide suppressed activation of LP-T via CD3 or CD2 (Fig. 1 A). Similarly, specific inhibition of the enzyme glutathione reductase by BCNU (11), leading to accumulation of oxidized glutathione, as well as specific inhibition of glutathione synthesis by BSO (12), abolished proliferation of LP-Ts. On the contrary, the reducing thiol compound 2-ME potentiated activation at concentrations as low as 5–10 μM. The antioxidant DTT enhanced proliferation reaching peak values at 0.5 mM and 1 mM for CD3 and CD2 stimulation, respectively (data not shown). Shifting the redox milieu from a prooxidant to a more antioxidative state restored proliferation again (Fig. 1 B): addition of 2-ME was able to counteract the suppression of T cell proliferation induced by hydrogen peroxide or BSO.

Evidence for a Redox-based Mechanism of Costimulation of LP-Ts by PB-MOs but Not LP-MOs. The proliferation of LP-Ts after stimulation with OKT3 alone was considerably enhanced by the addition of PB-MOs (Fig. 2 A). This costimulatory effect of PB-MOs increased up to 30% of total cell number. Similar results were obtained after CD2 stimulation. However, LP-MOs failed to support the proliferation of LP-Ts. The antioxidant 2-ME (10 μM) fully re-

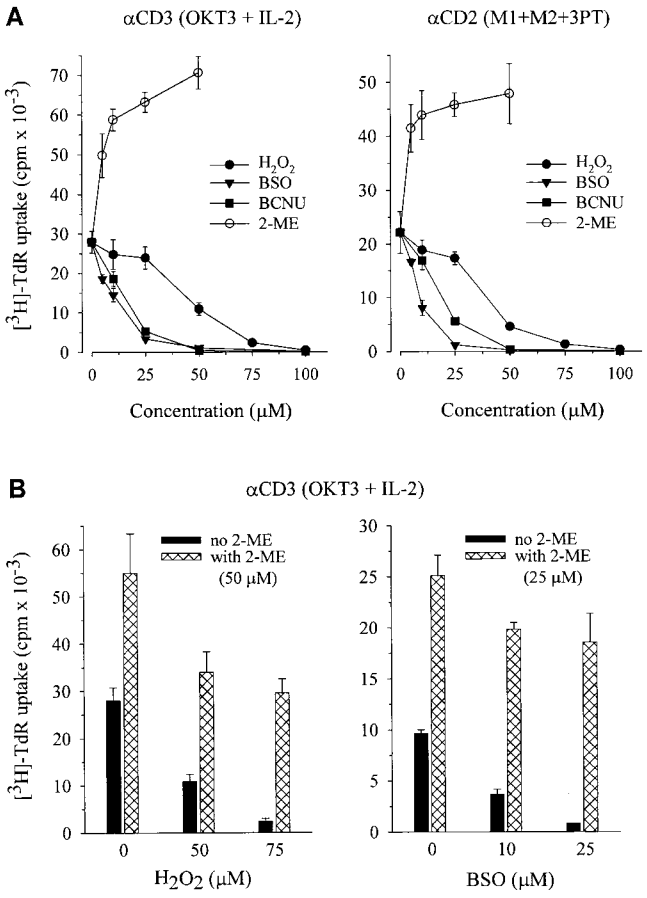

Figure 1. Redoxregulation of DNA synthesis in LP-Ts. (A) Influence of antioxidative (2-ME) and prooxidant (hydrogen peroxide, BSO, BCNU) culture conditions on the proliferation of LP-Ts after CD3 (10 U/ml IL-2 added) or CD2 stimulation. (B) Protective effect of 2-ME on the hydrogen peroxide or BSO-induced suppression of proliferation.

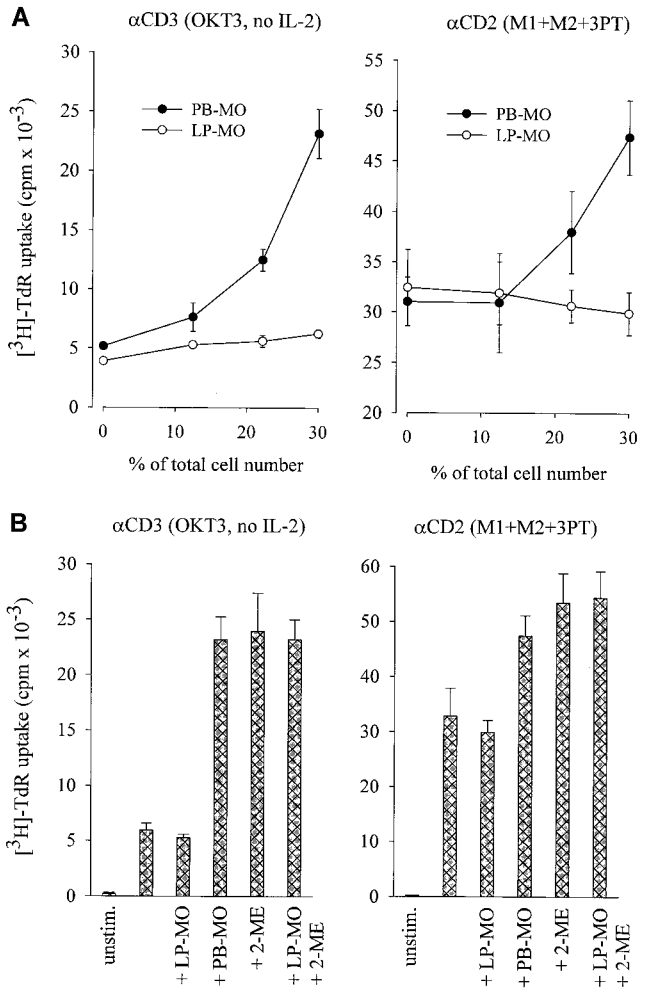

Figure 2. Costimulation of LP-Ts by PB-MOs or 2-ME, but not by LP-MOs. (A) Irradiated LP-MOs or autologous PB-MOs were added to LP-Ts (5×10^4/well) in graded amounts up to 30% of total cell number. (B) Costimulatory potential of 2-ME in comparison with irradiated PB-MOs and LP-MOs (30% of total cell number). unstim., unstimulated.

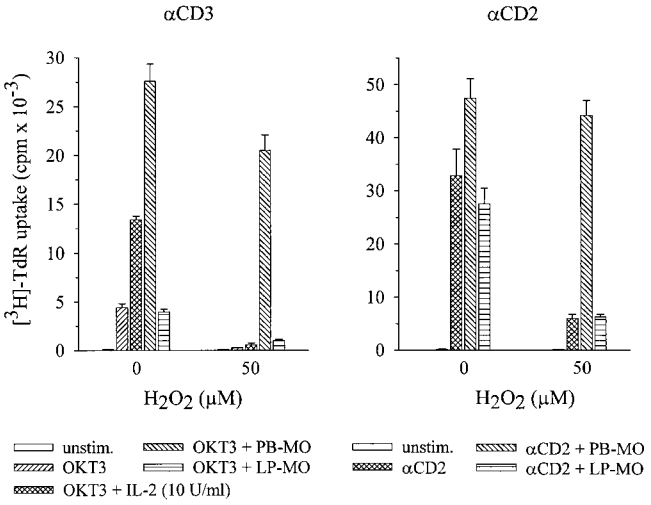

Figure 3. Protective effect of PB-MOs versus LP-MOs on the suppression of proliferation of LP-Ts by hydrogen peroxide, which was added at the beginning of the culture. unstim., unstimulated.

stored the CD3 reactivity of LP-T without any costimulus: proliferation was equivalent to cultures containing PB-MOs and LP-Ts (Fig. 2 B). Note that LP-MOs did not actively suppress proliferation. 2-ME enhances T lymphocyte proliferation by increasing uptake of cysteine derived from cystine (8), thus compensating for the low membrane transport activity of lymphocytes for cystine (10, 13). However, monocytes/macrophages readily take up cystine which, after intracellular reduction, is secreted as cysteine (14, 15). Thus, the proliferation of LP-T is subject to thiol-mediated redoxregulation, and release of reducing equivalents by PB-MOs but not by LP-MOs could be relevant for costimulation of LP-Ts. This view is supported by the fact that coculture of LP-Ts with PB-MOs abolished the suppressive effect of hydrogen peroxide, whereas LP-MOs completely lacked this capacity (Fig. 3).

PB-MO–mediated Costimulation of LP-Ts Involves CD2–CD58 Interaction. Does the redox-mediated costimulation of LP-Ts by PB-MOs involve receptor–ligand interactions? CD2–CD58 interactions could be a candidate, as the expression of CD58 is known to markedly differ between PB-MOs and LP-MOs (5). Disruption of CD2–CD58 interactions by saturating concentrations of the blocking CD58 mAb 1A3 (10 μg/ml) reduced the costimulatory effect of PB-MOs by 63% when proliferation of LP-Ts was induced by M1+M2 mAb (Fig. 4). The inhibitory action of 1A3 was on PB-MOs and not on lymphocytes, as stimulation induced by the mitogenic combination of the CD2 mAb M1+M2+3PT was not influenced by 1A3.

Cross-linking of CD58 on PB-MOs, but not on LP-MOs, Induces Release of Cysteine. We next explored the effect of CD58 cross-linking on thiol release by PB-MOs. To this end, PB-MOs (5×10^5/ml) were added to wells that were precoated with the CD58 mAb 1A3 (IgG2a). Release of cysteine was measured as acid-soluble thiol in the supernatant after 40 h of culture. All of the acid-soluble thiol in the supernatant could be defined as cysteine when analyzed by HPLC (16), and no GSH was detectable (data not shown). As shown in Fig. 5 A, PB-MOs constitutively released micromolar amounts of cysteine. Cross-linking of CD58 enhanced production three- to fourfold, equivalent to stimulation with LPS (1 μg/ml), whereas a CD54 mAb (Fig. 5 A) or an isotype-matched control antibody (not shown) were ineffective. Interestingly, IFN-γ (200 U/ml), a potent activator of PB-MOs, failed to enhance cysteine production in both unstimulated (Fig. 5 A) and LPS-treated PB-MOs (not shown). Note that LP-MOs did not release any cysteine into the supernatant, neither constitutively nor after activation with LPS or cross-linking of CD58 (Fig. 5 A). 2-ME (25 μM), inhibition of glutathione synthesis by BSO (25 μM), hydrogen peroxide (50 μM), and BCNU (25 μM) did not affect thiol production by PB-MOs (Fig. 5 B), suggesting that a redox system other than GSH/GSSG is involved in intracellular reduction of cystine to cysteine in PB-MOs.

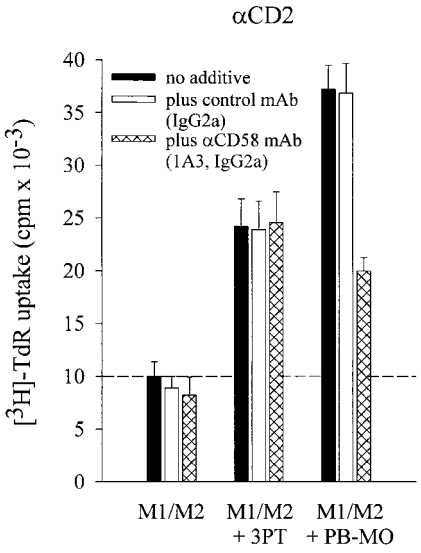

Figure 4. Involvement of CD2–CD58 interactions in PB-MO–mediated costimulation of LP-Ts. CD58 mAb (1A3) was used at saturating concentrations (10 μg/ml). Irradiated PB-MOs were added at 30% of total cell number. The dotted line indicates the baseline as defined by the proliferation induced by the CD2 mAb M1+M2.

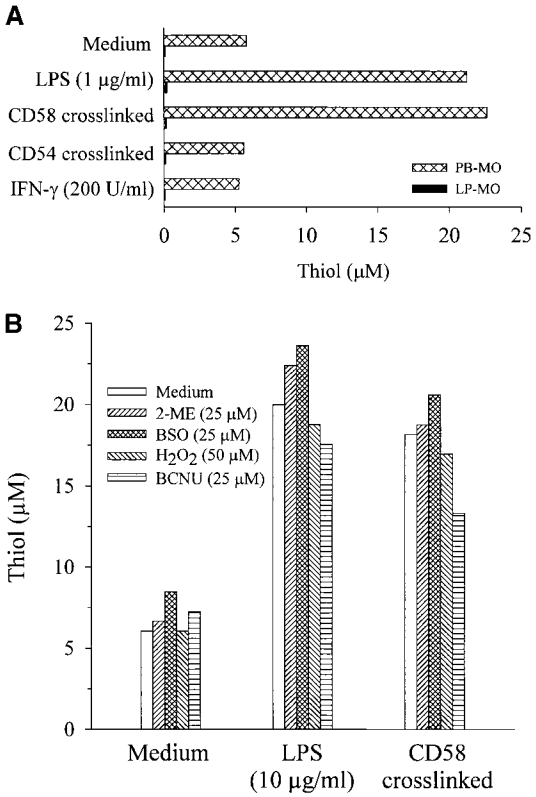

Figure 5. (A) Release of cysteine (acid-soluble thiol) by PB-MOs versus LP-MOs after 40 h of culture. Cells were plated at 5×10^5/ml in a total volume of 1 ml/well in 48-well culture plates, and were either left untreated or activated with LPS or IFN-γ. For cross-linking of CD58 and CD54, wells were precoated with 1A3 and GP89 mAb, respectively, at 10 μg/ml in PBS for 2 h at 37°C followed by two washes with PBS/5% FCS. Data represent one of three independent experiments performed with identical results. (B) Effect of 2-ME, hydrogen peroxide, BSO, and BCNU, respectively, on release of cysteine (acid-soluble thiol) by PB-MOs.

Thiol-mediated Redoxregulation of LP-Ts by PB-MOs. In contrast to lymphocytes, monocytes/macrophages have a high membrane transport activity for cystine (14). After intracellular reduction of cystine, exported cysteine is taken up by T cells (10, 14, 15, 17). It follows that costimulation of LP-Ts by PB-MOs (see Fig. 2) should be strictly dependent on the availability of cystine. Indeed, as shown in Fig. 6 A, the costimulatory potential of PB-MOs (or 2-ME) declined with decreasing concentrations of cystine.

If PB-MOs costimulate proliferation of LP-Ts by release of cysteine, then (in the absence of PB-MOs) supplementation of cysteine should result in a higher proliferation rate than equimolar amounts of cystine. To test this hypothesis, cystine-free cultures of LP-Ts were supplemented with either 30 μM cysteine or equimolar amounts of cystine (15 μM = 30 μM cysteine equivalents) every 6 h. Repetitive applications were necessary because cysteine is rapidly oxidized in culture to cystine with a half-life of ~1 h. Although the cumulative cystine concentration was 255 μM (510 μM cysteine equivalents) at termination of the culture, the presence of only 30 μM cysteine was far superior (Fig. 6 B). Transport of cysteine to lymphocytes is the limiting step in maintaining a critical intracellular GSH concentration (10, 17), which is crucial for cell cycle progression (7). As shown in Table I, supplementation of cystine-free cultures with GSH (3 mM) in the absence of PB-MOs could restore proliferation as well. Equimolar amounts of oxidized glutathione (1.5 mM GSSG) were without effect.

Discussion

Proliferation of T cells from the human intestinal lamina propria is subject to thiol-mediated redox regulation. No proliferation was observed in thiol-deficient culture medium, but it could be restored by supplementation with cysteine, which was far superior to supplementation with equimolar amounts of its oxidized form, cystine. In lymphocytes, cysteine is the limiting substrate for the synthesis of GSH (10, 17). T lymphocytes have a strong transport activity for cysteine (10, 13), but this is of only limited use, because cysteine circulates at very low concentrations (~10 μM [16]). The plasma concentration of cystine is much higher (140 μM cysteine equivalents), but the membrane transport activity for cystine in human lymphocytes is >10-fold lower than for cysteine (10, 13). GSH is essential for cell cycle progression, as GSH depletion in human PBMCs has been shown to inhibit transition from G_1 to S phase (7). Accordingly, proliferation of LP-Ts is inhibited in the presence of BSO and, conversely, the abortive CD3 and CD2 stimulation of LP-Ts in cystine-deficient medium is fully restored by administration of GSH. Antioxidative cellular redox conditions support lymphocyte proliferation, as we have shown with the use of 2-ME, DTT, and GSH, whereas prooxidant conditions in the presence of hydrogen

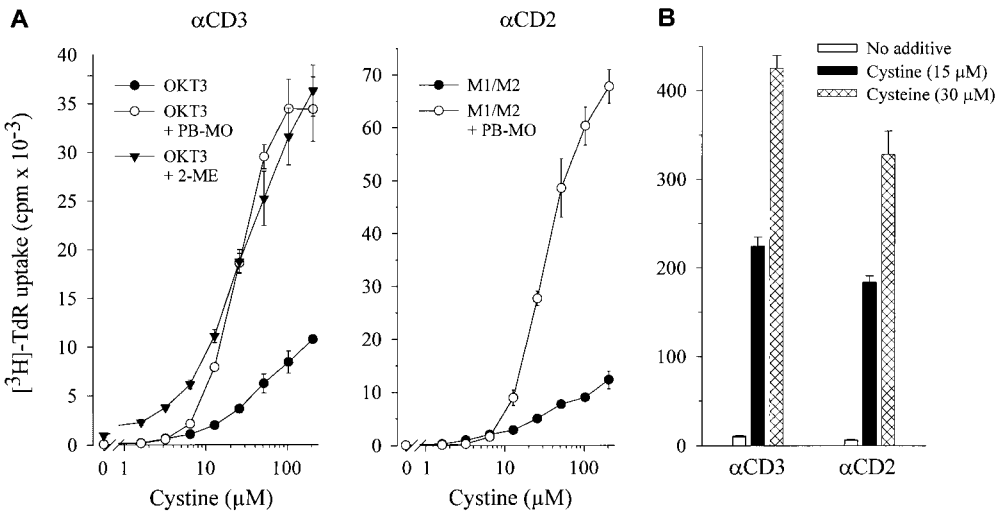

Figure 6. Thiol-dependent proliferation of LP-T. (A) Cystine-deficient RPMI 1640/10% FCS was supplemented with graded amounts of cystine at the indicated final concentrations. Concentration of 2-ME was 10 μM. Irradiated PB-MOs were added at 30% of total cell number. (B) Stimulatory potential of cysteine versus equimolar amounts of cystine on the proliferation of LP-Ts after CD3 (OKT3) or CD2 (M1+M2) stimulation. LP-Ts were plated at 8×10^5/well in an initial volume of 1 ml/well in 24-well plates. Cultures were primarily set up in cystine-deficient medium, and were supplemented with cysteine in 15-μl volumes every 6 h at a final concentration of 30 μM each time. Cysteine had to be added repeatedly because of rapid oxidation to cystine under culture conditions. Alternatively, cultures received equimolar amounts of cystine. After 84 h of culture, wells were pulsed with [^{3}H]-TdR at 5 μCi/ml, and cells were harvested 18 h later.

peroxide, BSO, and BCNU inhibit DNA synthesis. Alterations of the intracellular redox balance profoundly affect early signal transduction events in human T cells: a decrease of 10–30% in the intracellular GSH content in peripheral blood T lymphocytes has been shown to completely block the TCR-stimulated elevation of cytoplasmic intracellular free calcium ($[Ca^{2+}]_i$; reference 18). The redox balance GSH/GSSG is delicately regulated by the enzymes glutathione peroxidase, which oxidizes GSH to GSSG, and glutathione reductase, which catalyzes the reduction of GSSG to GSH. A shift of the redox balance GSH/GSSG towards a more prooxidant state by specific inhibition of the enzyme glutathione reductase by BCNU (11), or alternatively by supplementation of cystine-free cultures with GSSG, abolishes proliferation.

Addition of the antioxidant 2-ME to cystine-containing culture medium fully substitutes for the costimulatory capacity of PB-MOs. 2-ME acts through the formation of mixed disulfides with cysteine derived from extracellular cystine (8). The 2-ME–cysteine mixed disulfide can be easily transported to lymphocytes via the membrane transporter shared with leucine and phenylalanine (8). Upon internalization, this mixed disulfide requires intracellular reduction to liberate cysteine. In contrast to lymphocytes, mouse peritoneal macrophages were shown to have a strong membrane transport activity for cystine (14). Macrophages convert excess cystine to cysteine and secrete it (14, 15), thereby increasing the glutathione content and DNA synthesis in murine lymph node cells (15). Triggered in part by CD2–CD58 interactions, human PB-MOs also export cysteine. Our findings support the view that release of this single chemically defined thiol compound enables proliferation of TCR/CD3-activated LP-Ts. The redox system responsible for intracellular reduction of cystine in PB-MOs is not known. The GSH/GSSG system is not a likely candidate, as we detected the release of large amounts of cysteine even if glutathione synthesis and the enzyme glutathione reductase were inhibited by BSO (12) and BCNU (11), respectively.

Table I. *Influence of Reduced Versus Oxidized Glutathione on the Proliferation of LP-Ts in Cystine-deficient Medium*

	Unstim.	αCD3 (OKT3)	αCD2 (M1+M2+3PT)
LP-T	65 ± 16	83 ± 31	61 ± 11
LP-T plus GSH (3 mM)	298 ± 38	27,830 ± 2,241	56,143 ± 5,226
LP-T plus GSSG (1.5 mM)	72 ± 14	78 ± 20	90 ± 34

Unstim., unstimulated.

Cross-linking of CD58 on PB-MO, mimicking multivalent CD2 receptor binding, represents a signal for PB-MOs, but not for LP-MOs, to release cysteine. The action of the immobilized CD58 mAb was specific, as cross-linking of CD54 using a mAb of the same Ig isotype or an isotype-matched control antibody failed to deliver cysteine. Disruption of CD2–CD58 interactions decreased the stimulatory capacity of PB-MOs by >60%. This suggests that additional as yet unknown receptor–ligand interactions or cytokines other than IFN-γ could be involved.

Resident LP-MOs are not capable of releasing cysteine. This finding could be explained by their low level expression of cell surface receptors involved in cysteine production (CD14, CD58; reference 5). Alternatively, membrane transport activity for cystine, intracellular reduction to cysteine, and export of cysteine might be intrinsically downregulated in LP-MOs. Thus, LP-MOs in normal gut may contribute dually to "insufficient" costimulation of LP-Ts: signals via CD2 are decreased by the lack of CD58 expression on LP-MOs, which in turn results in insufficient cys-

teine delivery for T cells. The low level expression of CD14 on LP-MOs might also be important to secure local T cell hyporesponsiveness in normal gut. The finding that intestinal macrophages from normal gut, in contrast to macrophages isolated from inflamed tissue in IBD, show only minimal responsiveness to LPS as judged from production of IL-1β (19), supports this notion.

This study may relate to the pathogenesis of IBD, where macrophage populations in inflammatory sites with a different phenotype than resident LP-MOs exist. The former dominate in severely inflamed perivascular areas because of a sustained recruitment of PB-MOs (20, 21), and could facilitate increased T cell proliferation in IBD (22) by means of cysteine release. In contrast, resident LP-MOs in normal gut may present antigen to LP-Ts but only insufficiently deliver costimulatory signals, likely because of their inability to release cysteine in association with low level CD54 and CD58 expression (5). Under conditions of excessive antigenic stimulation, defective cysteine delivery by "nonprofessional" APCs results in hyporesponsiveness of lymphocytes in vivo, whereas a cysteine-producing variant cell line induces a successful immune response (23). Thus, the differential capacity of PB-MOs and LP-MOs to release cysteine evolves as a novel mechanism ensuring physiological hyporesponsiveness of LP-T despite continous antigen exposure in the normal intestinal microenvironment and, in turn, increased lymphocyte reactivity in IBD.

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References

1. Qiao, L., G. Schürmann, M. Betzler, and S.C. Meuer. 1991. Activation and signaling status of human lamina propria T lymphocytes. *Gastroenterology*. 101:1529–1536.
2. Targan, S.R., R.L. Deem, M. Liu, S. Wang, and A. Nel. 1995. Definition of a lamina propria T cell responsive state. Enhanced cytokine responsiveness of T cells stimulated through the CD2 pathway. *J. Immunol.* 154:664–675.
3. De Maria, R., S. Fais, M. Silvestri, L. Frati, F. Pallone, A. Santoni, and R. Testi. 1993. Continuous in vivo activation and transient hyporesponsiveness to TcR/CD3 triggering of human gut lamina propria lymphocytes. *Eur. J. Immunol.* 23:3104–3108.
4. Autschbach, F., J. Braunstein, B. Helmke, I. Zuna, G. Schürmann, Z.I. Niemir, R. Wallich, H.F. Otto, and S.C. Meuer. 1998. In situ expression of interleukin-10 in noninflamed human gut and in inflammatory bowel disease. *Am. J. Pathol.* 153:121–130.
5. Qiao, L., J. Braunstein, M. Golling, G. Schürmann, F. Autschbach, P. Möller, and S.C. Meuer. 1996. Differential regulation of human T cell responsiveness by mucosal versus blood monocytes. *Eur. J. Immunol.* 26:922–927.
6. Qiao, L., G. Schürmann, F. Autschbach, R. Wallich, and S.C. Meuer.1993. Human intestinal mucosa alters T cell reactivities. *Gastroenterology*. 105:814–819.
7. Messina, J.P., and D.A. Lawrence. 1989. Cell cycle progression of glutathione-depleted human peripheral blood mononuclear cells is inhibited at S phase. *J. Immunol.* 143:1974–1981.
8. Ishii, T., S. Bannai, and Y. Sugita. 1983. Mechanism of growth stimulation of L1210 cells by 2-mercaptoethanol in vitro. Role of the mixed disulfide of 2-mercaptoethanol and cysteine. *J. Biol. Chem.* 256:12387–12392.
9. Bull, D.M., and M.A. Bookman. 1977. Isolation and functional characterization of human intestinal mucosal lymphoid cells. *J. Clin. Invest.* 59:966–974.
10. Ishii, T., Y. Sugita, and S. Bannai. 1987. Regulation of glutathione levels in mouse spleen lymphocytes by transport of cysteine. *J. Cell. Physiol.* 133:330–336.
11. Frischer, H., and T. Ahmad. 1977. Severe generalized glutathione reductase deficiency after antitumor chemotherapy with BCNU [1,3-bis(chloroethyl)-1-nitrosourea]. *J. Lab. Clin. Med.* 89:1080–1091.
12. Griffith, O.W., and A. Meister. 1979. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl-homocysteine sulfoximine). *J. Biol. Chem.* 254: 7558–7560.
13. Gmünder, H., H.-P. Eck, and W. Dröge. 1991. Low membrane transport activity for cystine in resting and mitogenically stimulated human lymphocyte preparations and human T cell clones. *Eur. J. Biochem.* 201:113–117.
14. Watanabe, H., and S. Bannai. 1987. Induction of cystine transport activity in mouse peritoneal macrophages. *J. Exp. Med.* 165:628–640.
15. Gmünder, H., H.-P. Eck, B. Benninghoff, S. Roth, and W. Dröge. 1990. Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine. *Cell. Immunol.* 129:32–46.
16. Mansoor, M.A., A.M. Svardal, and P.M. Ueland. 1992. Determination of the in vivo redox status of cysteine, cysteinylglycine, homocysteine, and glutathione in human plasma. *Anal. Biochem.* 200:218–229.
17. Bannai, S., and N. Tateishi. 1986. Role of membrane transport in metabolism and function of glutathione in mammals. *J. Membr. Biol.* 89:1–8.
18. Staal, F.J.T., M.T. Anderson, G.E.J. Staal, L. Herzenberg, C. Gitler, and L. Herzenberg. 1994. Redox regulation of signal transduction: tyrosine phosphorylation and calcium influx. *Proc. Natl. Acad. Sci. USA*. 91:3619–3622.
19. Mahida, Y.R., K. Wu, and D.P. Jewell. 1989. Enhanced production of interleukin-1β by mononuclear cells isolated from mucosa with active ulcerative colitis or Crohn's disease. *Gut.* 30:835–838.
20. Rugtveit, J., P. Brandtzaeg, T.S. Halstensen, O. Fausa, and H. Scott. 1994. Increased macrophage subset in inflammatory bowel disease: apparent recruitment from peripheral blood monocytes. *Gut.* 35:669–674.
21. Burgio, V.L., S. Fais, M. Boirivant, A. Perrone, and F. Pallone. 1995. Peripheral monocyte and naive T-cell recruitment and activation in Crohn's disease. *Gastroenterology*. 109: 1029–1038.
22. Autschbach, F., G. Schürmann, L. Qiao, H. Merz, R. Wallich, and S.C. Meuer. 1995. Cytokine messenger-RNA expression and proliferation status of intestinal mononuclear cells in noninflamed gut and Crohn's disease. *Virchows Arch.* 426:51–60.
23. Roth, S., and W. Dröge. 1994. Glutathione reverses the inhibition of T cell responses by superoptimal numbers of "nonprofessional" antigen presenting cells. *Cell. Immunol.* 155:183–194.