CD2 activation of human lamina propria lymphocytes reduces CD3 responsiveness

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Summary

Lamina propria lymphocytes (LPLs) are thought to be antigen-activated memory T cells. Yet, they respond better to ligation of the CD2 receptor than the CD3 receptor by mitogenic antibodies. This study defines their constitutive state of activation and relates it to their CD3 hyporesponsiveness. The activated state of LPLs was demonstrated by their heightened display of the activated CD2 epitope, $T11_3$. Constitutive CD2 activation was shown by the reduction in spontaneous proliferation when the CD2–CD58 interaction was blocked. LPLs preferentially recognized CD58 rather than the major histocompatibility complex molecules on monocytes, triggering proliferation and interferon-γ (IFN-γ) secretion that was inhibited by blocking the CD2–CD58 interaction. To determine whether CD2 activation of LPLs contributes to their CD3 hyporesponsiveness, they were first stimulated with mitogenic CD2 antibodies and then tested for CD3-induced proliferation. The responses were greatly reduced by prior CD2 stimulation compared with LPLs cultured in medium alone. This effect was not caused by apoptosis or by changes in CD3 expression induced by CD2 triggering. This study shows that LPLs are constitutively activated through CD2, that they preferentially recognize CD58 on monocytes and that CD2 stimulation leads to CD3 hyporesponsiveness.

Keywords: CD2; CD3; lamina propria lymphocytes; interferon-gamma

Introduction

CD2, a highly conserved molecule in primates, is found on both T cells and natural killer (NK) cells. It is composed of several epitopes: $T11_1$, the sheep red blood cell-binding site; $T11_2$, an epitope with similar distribution; and $T11_3$, an epitope expessed with T-cell activation.[1] The main ligand in humans is CD58, a marker found at higher densities on monocytes than on either B cells or non-haematological cells. Ligation of $T11_2$ and $T11_3$ on naïve T cells with mitogenic antibodies results in proliferation and responsiveness to interleukin (IL)-1 and IL-12.[1–3] CD2 may serve to enhance T-cell receptor-αβ (TCR-αβ)-mediated responses to low concentrations of antigen by positioning the T-cell/antigen-presenting cell (APC) membrane at a distance optimal for TCR-αβ engagement of major histocompatibility complexes (MHCs), perhaps by excluding larger proteins, such as CD45, or by recruiting signalling molecules towards the TCR-αβ complex.[4–7] In contrast, stimulation through the CD2 pathway can down-regulate the TCR-αβ response, leading to T-cell unresponsiveness or tolerance.[8–11]

Lamina propria lymphocytes (LPLs) express antigens associated with acute activation, such as CD25 and CD69, as well as those associated with chronic activation, such as CD45RO. This compartment of lymphocytes is probably derived from Peyer's patch lymphocytes that have been stimulated by foreign antigens. Once in the intestine,

Abbreviations: APC, antigen-presenting cell; c.p.m., counts per minute; FITC, fluorescein isothiocyanate; IEL, intraepithelial lymphocyte; IFN-γ, interferon-γ; IL, interleukin; LPL, lamina propria lymphocyte; mAb, monoclonal antibody; MHC, major histocompatibility complex; PB, peripheral blood; PBL, peripheral blood lymphocytes; TCR, T-cell receptor; TNF-α, tumour necrosis factor-α.

they may be exposed to antigens that pass through or between intestinal epithelial cells to enter the lamina propria. This scenerio implies that LPLs are chronically activated through the CD3/TCR-αβ complex. Yet, they are incompletely activated through this pathway, demonstrating minimal to absent calcium ion mobilization and phospholipid turnover.[12–18] In contrast, they display strong responsiveness to ligation of $T11_2$ and $T11_3$ with mitogenic antibodies, associated with the release of interferon-γ (IFN-γ), IL-2, IL-4 and tumour necrosis factor-α (TNF-α). Human intestinal intraepithelial lymphocytes (IEL) also have a stronger response to stimuli of the CD2 rather than the CD3 receptor,[19] indicating that this is a property of all mucosal lymphocytes.

The mechanism responsible for the constitutive activation of LPLs is unknown. This activity may be a good reflection of events *in situ*. The present study demonstrates that constitutive proliferation is mediated through CD2 and that CD2 stimulation results in activation-induced CD3 hyporesponsiveness.

Materials and methods

Isolation of lymphocytes

LPLs were separated from jejunal mucosa obtained from healthy subjects undergoing gastric bypass operations for morbid obesity.[19] Briefly, the minced mucosa was treated with 1 mM dithiothreitol (DTT) (Sigma Chemical Co., St Louis, MO) for 30 min at 37°, followed by six 45-min treatments with 0·75 mM EDTA (Sigma) to remove IELs and epithelial cells. The remaining tissue was digested for 3 hr in 20 U/ml collagenase (Worthington Biochemical Corporation, Lakewood, NJ), then pressed through a wire mesh sieve to release the tissue-bound cells. Purification on a Percoll density gradient (Pharmacia, Piscataway, NJ) yielded LPLs that were 98 ± 5% $CD2^+$, 88 ± 5% $CD3^+$, 56 ± 10% $CD4^+$, 35 ± 11% $CD8^+$, 0 ± 1% $CD14^+$, 2 ± 1% $CD16^+$, 1 ± 0% $CD19^+$ and 1 ± 2% $CD58^+$.

Peripheral blood lymphocytes (PBLs) were isolated from whole blood by Ficoll density-gradient centrifugation. T cells were obtained by removing monocytes and B cells using immunomagnetic separation with CD14 and CD19. To obtain monocytes, the PBLs were first irradiated (4000 rads), then layered in microwells. After 45 min at 37°, the non-adherent cells were removed, leaving $\approx 2 \times 10^4$ monocytes per microwell. LPLs were added to these wells (2×10^5/0·1 ml) in co-culture experiments.

Proliferation and cytokine secretion

Lymphocytes were cultured in medium alone, with antibody to CD3-ε (1 μg/ml; Coulter-Immunotech, Miami, FL), or with the mitogenic CD2 antibody pair, $T11_2$ and $T11_3$ (1 : 500 dilution, a gift from E. Reinherz, Dana-Farber Cancer Institute, Boston, MA). Specific receptor interactions were blocked by CTLA4–Fc (R & D Systems, Minneapolis, MN) or by monoclonal antibodies (mAbs) (5 μg/ml) recognizing the following: $OKT11_1$ (Ortho Diagnostics, Raritan, NJ), CD25, CD58, CD80, CD86, IL-2 receptor, MHC classes I and II (HLA-DR) (all from Coulter-Immunotech), IL-1β, IL-2, IL-6, or TNF-α (all from R & D Systems). Proliferation was measured by a 6-hr pulse of 1 μCi [^{3}H]thymidine (6·7 Ci/mmole; ICN Biomedicals, Costa Mesa, CA). Cytokine secretion was measured in culture supernates by specific enzyme-linked immunosorbent assays (ELISAs) (R & D Systems).

Immunofluorescence

Lymphocytes were labelled directly by fluorescein-conjugated mAbs, or indirectly by mAb engagement followed by goat anti-mouse immunoglobulin G (IgG) conjugated to fluorescein isothiocyanate (FITC). The antibodies used included those listed above as well as antibody recognizing TCR-αβ (R & D Systems). Analysis by flow cytometry included the percentage of positively stained cells as well as the intensity of expression, described as the relative fluorescence intensity (RFI) or the mean channel number with the test antibody divided by the mean channel number with the secondary reagent alone.

Statistics

The mean and standard deviation (SD) of the test values were compared using the Student's *t*-test or the Wilcoxon Rank Sum Test for paired or independent variables.

Results

Previous studies have shown that LPLs react to anti-$T11_2$ and anti-$T11_3$ to the same degree as naïve T cells. However, they have a blunted response to CD3 ligation. The question presented in this study is whether their constitutively activated state involves the CD3 pathway (like antigen-activated T cells) or the CD2 pathway.

Initial studies evaluated the expression of CD2 and CD3 on LPLs and peripheral blood (PB) T cells by immunofluorescence and flow cytometric analysis. As LPLs have been found to express markers of acute activation (CD25 and HLA-DR), they may also display epitopes of CD2 associated with activation, specifically $T11_3$. To evaluate this, LPLs and PB T cells were stained by immunofluorescence for the three epitopes of CD2. Fresh LPLs displayed higher densities of both $T11_2$ and $T11_3$ when compared with PB T cells (Fig. 1). The expression of $T11_1$ also tended to be higher on LPLs than on PB T cells, but this did not reach statistical significance. CD3 and TCR-αβ expression, on the other hand, was found at the same density on the two cell types.

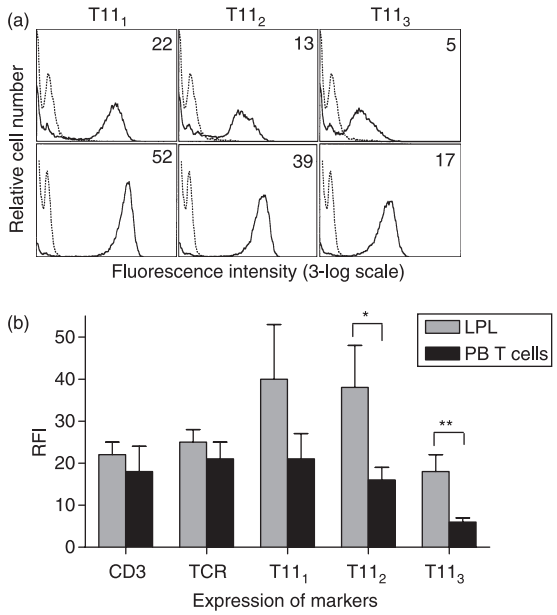

Figure 1. Expression of $T11_1$, $T11_2$ and $T11_3$ on peripheral blood lymphocytes (PBLs) and lamina propria lymphocytes (LPLs). (a) Freshly isolated autologous peripheral blood (PB) T cells (upper panels) and LPLs (lower panels) were stained by indirect immunofluorescence for three epitopes of CD2 and analysed by flow cytometry. The dotted lines depict negative-control cells stained with goat anti-mouse immunoglobulin G–fluorescein isothiocyanate (GAM–FITC) alone. Relative fluorescence intensity (RFI) in comparison to the negative control is found in the upper right hand corner of each graph. This is a representative result of six experiments. (b) The average RFI values for expression of surface markers are depicted ($^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $n = 6$).

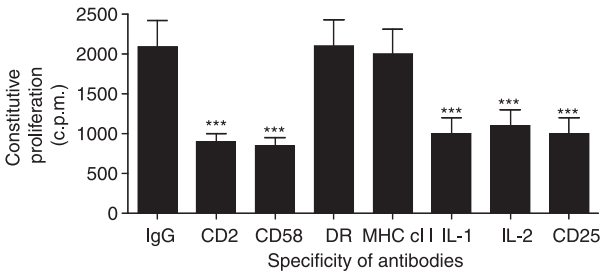

Figure 2. Effects of blocking antibodies on constitutive proliferation. Lamina propria lymphocytes (LPLs) were cultured in the presence of blocking antibodies. Proliferation was determined after 3 days by measuring [^{3}H]thymidine incorporation ($n = 23$). c.p.m., counts per minute; IgG, immunoglobulin G; IL, interleukin; MHC, major histocompatibility complex. $^{***}P < 0{\cdot}001$.

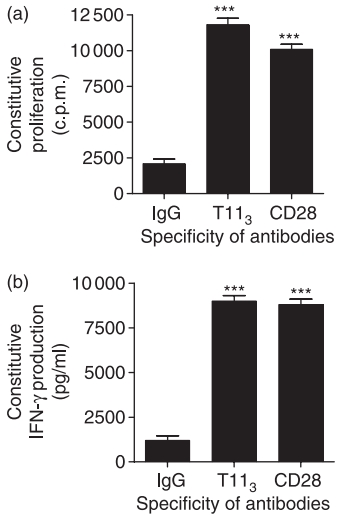

Figure 3. Effects of anti-$T11_3$ and anti-CD28 on proliferation and interferon-γ (IFN-γ) production. Lamina propria lymphocytes (LPLs) were cultured in the presence of stimulatory monoclonal antibodies (mAbs). Proliferation was determined after 3 days ($n = 8$) (a) and IFN-γ production was determined after 18 hr ($n = 5$) (b). IgG, immunoglobulin G. $^{***}P < 0{\cdot}001$.

Besides phenotypic evidence of activation, LPLs have functional evidence of activation, namely spontaneous proliferation and IFN-γ production when cultured in medium alone (Figs 2 and 3). Proliferation of LPLs in medium alone averaged 2094 ± 323 counts per minute (c.p.m.) ($n = 20$), peaking at 72 hr. This level of proliferation was 10-fold greater than the spontaneous proliferation of PB T cells (201 ± 53 c.p.m., $n = 9$), and 19-fold more than the background obtained with irradiated LPLs (113 ± 88 c.p.m., $n = 4$). Spontaneous IFN-γ production was 1200 ± 200 pg/ml, compared with less than 30 pg/ml for PB T cells.

Functional evidence of an activated state was also demonstrated by the marked up-regulation of proliferation and IFN-γ production by LPLs in response to either anti-$T11_3$ or anti-CD28[20] (Fig. 3). PB T cells, in contrast, showed minimal spontaneous proliferation and IFN-γ production and no up-regulation with either antibody alone. Rather, ligation of both $T11_2$ and $T11_3$ is required for a functional response.

To determine whether spontaneous events were mediated through CD2, constitutive proliferation by LPLs was measured in the presence of antibodies blocking CD2, or its ligand, CD58 (Fig. 2). Spontaneous LPL proliferation decreased upon incubation in the presence of antibodies blocking either CD2 ($T11_1$) or CD58, but not upon incubation with antibodies blocking HLA-DR or MHC class I, the ligands for the CD3/TCR-αβ complex. Such constitutive proliferation required IL-1, IL-2 and CD25 as it was reduced by antibodies blocking these molecules. Constitutive IFN-γ production decreased in five out of eight experiments upon the addition of anti-CD58 – a suggestive but non-significant result.

Next, studies were conducted to determine the markers on APCs preferentially recognized by LPLs – the ligands for CD2 or those for CD3/TCR-αβ (CD58 or MHC class I and II, respectively). To distinguish between spontaneous and monocyte-induced events, LPLs were first

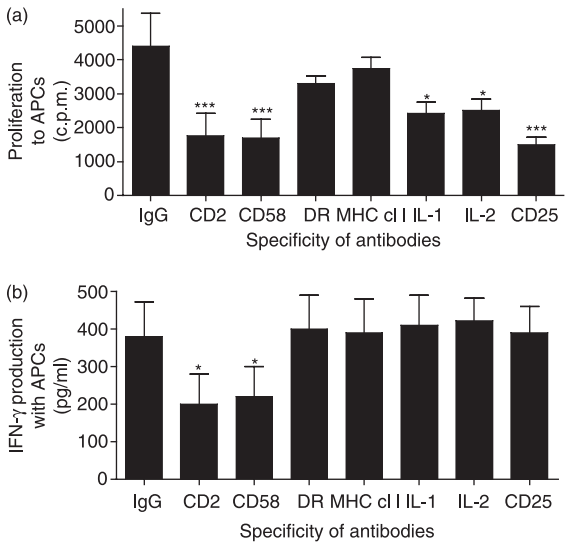

Figure 4. Effects of blocking antibodies on proliferation and interferon-γ (IFN-γ) production in response to antigen-presenting cells (APCs). Lamina propria lymphocytes (LPLs) were cultured for 3 days in medium alone. Then, they were exposed to autologous APCs in the presence of blocking monoclonal antibodies (mAbs). Proliferation was measured by determining the [^{3}H]thymidine incorporation after an additional 3 days ($n = 8$) (a) while IFN-γ production was measured after 18 hr ($n = 6$) (b). c.p.m., counts per minute; IgG, immunoglobulin G; IL, interleukin; MHC, major histocompatibility complex. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$.

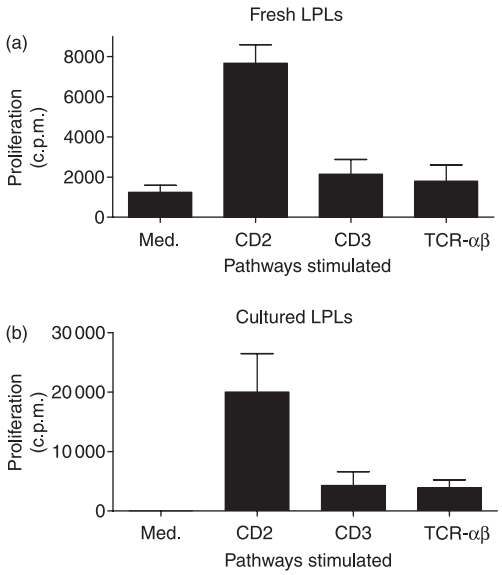

Figure 5. Proliferation of fresh and cultured lamina propria lymphocytes (LPLs) in response to CD2 or CD3 ligation. LPLs were tested just after isolation (a, fresh LPLs) or after a 3-day culture in medium (b, cultured LPLs). The cells were stimulated with antibodies ligating CD2 ($T11_2$ and $T11_3$), CD3, or T-cell receptor-αβ (TCR-αβ), and proliferation was measured after a 3-day culture ($n = 15$). c.p.m., counts per minute; Med., medium.

cultured in medium alone for 3 days during which time the spontaneous activity subsided. However, they still expressed the same density of $T11_3$ on their surface as they did just after isolation ($n = 3$, data not shown). They were then cultured (2×10^5/0·1 ml) with irradiated autologous PB monocytes (2×10^4/0·1 ml) in the presence or absence of blocking mAbs. Incorporation of [^{3}H]thymidine was measured after an additional 3-day incubation, a duration found in preliminary kinetics studies to yield the greatest response (Fig. 4). LPL proliferation increased from 230 ± 109 c.p.m. without monocytes to 4400 ± 987 c.p.m. with monocytes ($P < 0{\cdot}01$, $n = 6$), a response that was strongly inhibited by mAbs blocking CD2 or CD58, but not by mAbs blocking DR or MHC class I. Like constitutive proliferation, blocking IL-1 or IL-2 reduced cell division in response to APCs, and blocking CD25 had a particularly strong inhibitory effect. In order to assess IFN-γ production, LPLs were cultured in medium for 3 days, then exposed to autologous monocytes; medium was collected after 18 hr and tested for IFN-γ content. The amount of IFN-γ produced with APCs (380 ± 93 pg/ml) greatly exceeded that produced with medium alone (25 ± 8 pg/ml, $P < 0{\cdot}001$). Blocking CD2 or CD58 reduced IFN-γ production, while blocking DR or MHC class I had no effects. This action was not dependent on IL-2, CD25, or IL-1.

As demonstrated previously,[12–18] LPLs have substantially greater proliferative responses to CD2 than to CD3/TCR-αβ cross-linking, the latter being non-significantly different in this study from proliferation in medium alone (fresh LPLs, Fig. 5). LPLs, cultured in medium for 3 days, were retested for their responses to receptor ligation. Again, CD2 responses were greater than CD3 or TCR-αβ responses (cultured LPLs, Fig. 5). However, with spontaneous proliferation gone, there was now a significant difference between medium and CD3- or TCR-αβ-induced cell division. In addition, the proliferative responses were much greater overall, except for the medium control. This suggests that fresh LPLs have minimal to no CD3/TCR-αβ responsiveness, perhaps as a result of their constitutive CD2 activation. When this chronic stimulation abates during culture in medium alone, LPLs were able to mount some CD3/TCR-αβ response, but not to the levels reached with CD2 ligation.

The dependence of CD2- and CD3-induced proliferation on surface markers and cytokines was determined by adding blocking antibodies, as in Figs 2 and 4. When proliferation was induced by mitogenic mAbs recognizing $T11_2$ and $T11_3$, antibody blocking $T11_1$ had a modest inhibitory effect. It did not affect proliferation induced by CD3 cross-linking (Fig. 6). No changes in CD2- or CD3-induced proliferation occurred with antibodies obstructing CD58, DR, or MHC class I. This suggests that the inhibitory effects of anti-$T11_1$ or anti-CD58 on

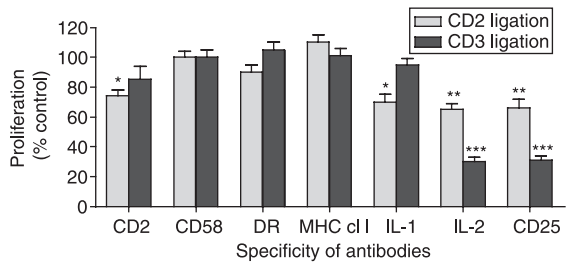

Figure 6. Effects of blocking antibodies on proliferation caused by CD2 or CD3 ligation. Lamina propria lymphocytes (LPLs) were cultured for 3 days with the mitogenic antibody pair recognizing $T11_2$ and $T11_3$, or with anti-CD3, each in the presence of blocking antibodies. Proliferation averaged 18 244 counts per minute (c.p.m.) for the mitogenic CD2 antibodies [similar to peripheral blood (PB) T cells] while the proliferation averaged 3544 c.p.m. for CD3 (15 077 c.p.m. for PB T cells). Proliferation was determined by measuring the [^{3}H]thymidine incorporation ($n = 8$). IL, interleukin; MHC, major histocompatibility complex. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$.

spontaneous and monocyte-induced functions are not caused by a direct action of the antibodies on the LPLs. Stimulation with the mitogenic CD2 antibody pair does not require CD58. CD2-induced proliferation was partially reduced by blocking IL-1, IL-2 or CD25, similarly to spontaneous proliferation (Fig. 3). CD3-mediated proliferation was independent of IL-1, but markedly dependent on IL-2 and CD25 (Fig. 6).

As LPLs are more prone to apoptosis than naïve T cells, the differences between the CD2 and CD3 responses may be related to differences in survival. This was tested by staining with annexin and propidium iodide (PI), by investigating apo 2.7 expression, and by cell cycle analysis. With each technique, no differences were found in the numbers of apoptotic and necrotic cells, regardless of whether the LPLs were stimulated through the CD2 or CD3 pathways or whether they were cultured in medium alone. To test the survival of cultured cells, the LPLs were incubated for 3 days with medium or the mitogenic antibodies against CD2 or CD3. They were restimulated with medium, anti-CD2, or anti-CD3 for an additional 3 days. Again, there were no significant differences in apoptosis or necrosis among the groups.

CD2 activation may cause LPLs to become temporarily refractory to further stimulation. To test this possibility, LPLs were stimulated for 3 days with the combination of $T11_2$ and $T11_3$ mAbs, with anti-CD3, or with an IgG control. Cells were then washed and placed in a secondary culture for 3 days with one of the stimuli. This culture period was found to yield maximal proliferation in preliminary kinetics experiments. Activation through CD2 caused LPLs to proliferate extremely poorly in response to CD3 ligation in the secondary culture (Table 1). Prestimulation with anti-CD3 also reduced the subsequent CD2 response, although the effect was not as marked.

Table 1. CD2 activation induces CD3 hyporesponsiveness in lamina propria lymphocytes (LPLs), but not in peripheral blood (PB) T cells

Cell type	Stimulus in primary culture	Stimulus in secondary culture: IgG	Anti-CD2	Anti-CD3	IL-2
LPL	IgG control	0·1 ± 0	18·6 ± 5·6*	7·1 ± 1·1**	15·5 ± 2·5
	Anti-CD2	0·1 ± 0	1·4 ± 0·6	0·5 ± 0·1**	21·5 ± 2·2
	Anti-CD3	0·1 ± 0	5·2 ± 2·1*	0·2 ± 0·1	14·9 ± 1·2
PB T cell	IgG control	0·1 ± 0	21·3 ± 3·3	18·4 ± 3·2	8·5 ± 2·3
	Anti-CD2	0·1 ± 0	1·1 ± 2·1	15·4 ± 2·2	11·4 ± 2·2
	Anti-CD3	0·1 ± 0	18·8 ± 2·5	1·1 ± 1·0	10·5 ± 1·9

LPLs or PB T cells were cultured for 3 days with mitogenic monoclonal antiodies (mAbs) against CD2 or CD3 or with an immunoglobulin G (IgG) control. After washing, the lymphocytes were recultured with each stimulus for another 3 days. Proliferation was determined by [^{3}H]thymidine incorporation. The CD3 response in the secondary culture was reduced by prior CD2 stimulation ($^{**}P < 0{\cdot}01$). The CD2 response was reduced by prior CD3 stimulation, although to a much lesser extent ($^*P < 0{\cdot}05$, $n = 6$).
Data are expressed as proliferation, in counts per minute (c.p.m.) $\times 10^3$.

IL-2 responsiveness was similar, regardless of the treatment. In contrast, PB T cells proliferated well in the secondary culture, despite prestimulation with mitogenic mAbs. These findings were not the result of changes in apoptosis or necrosis, or of changes in CD3 expression induced by CD2 ligation.

Discussion

This study shows that LPLs are constitutively activated through CD2, and that CD2 stimulation reduces CD3 responses. The state of activation of LPLs is demonstrated by their expression of $T11_3$, which is not present on resting naïve T cells. Their spontaneous proliferation declines by blocking CD58 with specific antibody, suggesting that this activity is CD2-mediated. LPLs recognize and proliferate in response to CD58, rather than MHC antigens on monocytes, supporting a preferential activation through CD2 rather than CD3. Finally, stimulating LPLs through CD2 causes a marked impairment in their ability to proliferate in response to CD3 antibody. This was not the result of increased apoptosis or changes in CD3 expression mediated by CD2 cross-linking. Rather, this may be caused by activation-induced hyporesponsiveness.

LPLs have increased expression of $T11_2$ and $T11_3$ compared with naïve T cells. This heightened CD2 display may facilitate the recognition of CD58 on APCs, overcoming the traditional CD3/TCR-αβ–MHC class II interaction. To show a functional effect, the CD2–CD58 interaction was blocked, decreasing proliferation that occurs both spontaneously and in response to peripheral blood APCs. CD2 reactivity may be augmented by APCs,

as demonstrated by the development of CD2-reactive T cells after contacting Daudi cells;[13] conversely, reactivity to APC may be augmented by heightened CD2 expression. The monocyte product, IL-1, is involved in LPL proliferation occurring with medium alone, with APCs and with CD2 ligation, all mediated through CD2, but not with CD3 ligation. Similarly, IL-1 responsiveness of naïve T cells is facilitated by CD2 signalling.[2]

Few APCs are extracted with purified LPLs as they may adhere to the fibrous network of the mucosa. Their expression of CD14, CD80 and CD86 are low,[21] while their expression of CD58 is rarely evaluated. This study shows that normal LPLs have a small population of $CD58^+$ cells, perhaps responsible for triggering the constitutive CD2 activation. Crohn's disease is characterized by an infiltration of APCs from the PB. This influx may explain the CD2 hyper-responsiveness and granuloma formation seen in this disease.[15,22] CD58 is also found on the basolateral surfaces of epithelial cells where it can stimulate the CD2 pathway of $CD4^+$ T-cell clones[23] and perhaps also of LPLs.

This study shows that CD3-induced proliferation by LPLs is equivalent to that in medium alone. However, after culture in medium, subsequent stimulation through CD3 increases, although it is still lower than that of naïve T cells. These findings agree with the reported minimal-to-absent LPL proliferation, calcium influx and inositol triphosphate generation[1,4,5] that are partially restored after LPLs are cultured for at least 18 hr.[12] The increased CD3-induced responses with time in culture may be caused by a dissipation of the constitutive CD2 activation, which may block activities mediated through CD3.

This study shows that CD2 stimulation may contribute to the known incomplete CD3 responsiveness by LPLs. This is demonstrated by cross-linking CD2 with mitogenic mAbs, and, after 3 days, ligating CD3. The resulting CD3-induced proliferation is substantially reduced. This finding is not the result of cell death, apoptosis, or a reduction in CD3 expression by CD2 ligation. An initial stimulation with CD3 reduces CD2 responses to a lesser extent. It is probable that the greater the initial response, the greater the subsequent suppression caused by a prolonged refractory period not seen with PB T cells. CD2 activation may also be important in the suppression of TCR-mediated responses in the intestinal mucosa.

Acknowledgement

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References

1 Meuer SC, Hussey RE, Fabbi M *et al.* An alternative pathway of T-cell activation: a functional role for the 50 kd T11 sheep erythrocyte receptor protein. *Cell* 1984; **36**:897–906.

2 Endler-Jobst B, Schraven B, Hutmacher B, Meuer SC. Human T cell responses to IL-1 and IL-6 are dependent on signals mediated through CD2. *J Immunol* 1991; **146**:1736–42.

3 Gollob JA, Li J, Kawasaki H, Daley JF, Groves C, Reinherz EL, Ritz J. Molecular interaction between CD58 and CD2 counterreceptors mediates the ability of monocytes to augment T cell activation by IL-12. *J Immunol* 1996; **157**:1886–93.

4 Bachmann MF, Barner M, Kopf M. CD2 sets quantitative thresholds in T cell activation. *J Exp Med* 1999; **190**:1383–91.

5 van der Merwe P. Commentary. A subtle role for CD2 in T cell antigen recognition. *J Exp Med* 1999; **190**:1371–4.

6 Germain RN. Minireview: The T cell receptor for antigen: signaling and ligand discrimination. *J Biol Chem* 2001; **276**:35223–6.

7 Hahn WC, Rosenstein Y, Calvo V, Burakoff SJ, Bierer BE. A distinct cytoplasmic domain of CD2 regulates ligand avidity and T-cell responsiveness to antigen. *Proc Natl Acad Sci, USA* 1992; **89**:7179–83.

8 Guckel G, Berek C, Lutz M, Altevogt P, Schirrmacher V, Kyewski BA. Anti-CD2 antibodies induce T cell unresponsiveness *in vivo*. *J Exp Med* 1991; **174**:957–67.

9 Chavin KD, Qin L, Yon R, Lin J, Yagita H, Bromberg JS. Anti-CD2 mAbs suppress cytotoxic lymphocyte activity by the generation of Th2 suppressor cells and receptor blockade. *J Immunol* 1994; **152**:3729–39.

10 Chavin KD, Qin L, Lin J, Yagita H, Bromberg S. Combined anti-CD2 and anti-CD3 receptor monoclonal antibodies induce donor-specific tolerance in a cardiac transplant model. *J Immunol* 1993; **151**:7249–59.

11 Miller GI, Hochman PS, Meier W, Tizard R, Bixler SA, Rosa MD, Wallner BP. Specific interaction of lymphocyte function-associated antigen 3 with CD2 can inhibit T cell responses. *J Exp Med* 1993; **178**:211–22.

12 DeMaria R, Fais S, Silvestri M, Frati L, Pallone F, Santoni A, Testi R. Continuous *in vivo* activation and transient hyporesponsiveness to TCR/CD3 triggering of human gut lamina propria lymphocytes. *Eur J Immunol* 1993; **23**:3104–8.

13 Targan SR, Deem RL, Liu M, Wang S, Nel A. Definition of a lamina propria T cell responsive state: enhanced cytokine responsiveness of T cells stimulated through the CD2 pathway. *J Immunol* 1995; **154**:664–75.

14 Qiao L, Schurmann G, Betzler M, Meuer SC. Down-regulation of protein kinase C activation in human lamina propria T lymphocytes: influence of intestinal mucosa on T cell reactivity. *Eur J Immunol* 1991; **21**:2385–9.

15 Gonsky R, Deem RL, Hughes CCW, Targan SR. Activation of the CD2 pathway in lamina propria T cells up-regulates functionally active AP-1 binding to the IL-2 promoter, resulting in messenger RNA transcription and IL-2 secretion. *J Immunol* 1998; **160**:4914–22.

16 Gonsky R, Deem RL, Bream JH, Lee DH, Young HA, Targan SR. Mucosa-specific targets for regulation of IFN-γ expression. *J Immunol* 2000; **164**:1399–407.

17 Boirivant M, Pica R, DeMaria R, Testi R, Pallone F, Strober W. Stimulated human lamina propria T cells manifest enhanced Fas-mediated apoptosis. *J Clin Invest* 1996; **98**:2616–22.

18 Pirzer UC, Schuermann G, Post S, Betzler M, Meuer SC. Differential responsiveness to CD3-Ti vs CD2-dependent activation of human intestinal T lymphocytes. *Eur J Immunol* 1990; **20**:2339–42.

19 Ebert EC. Proliferative responses of human intraepithelial lymphocytes to various T-cell stimuli. *Gastroenterology* 1989; **97**:1372–81.
20 Zhou X-Y, Yashiro-Ohtani Y, Nakahira M *et al.* Molecular mechanisms underlying differential contribution of CD28 versus non-CD28 costimulatory molecules to IL-2 promoter activation. *J Immunol* 2002; **168**:3847–54.
21 Smith PD, Smythies LE, Mosteller-Barnum M *et al.* Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities. *J Immunol* 2001; **167**:2651–6.
22 L Mishra BB, Harris M, Bayless TM, Muchmore AV. *In vitro* cell aggregation and cell adhesion molecules in Crohn's disease. *Gastroenterology* 1993; **104**:772–9.
23 Framson PE, Cho DH, Lee LY, Hershberg RM. Polarized expression and function of the costimulatory molecule CD58 on human intestinal epithelial cells. *Gastroenterology* 1999; **116**:1054–62.