Autoimmunity, 2010; Early Online: 1–11

ISSN 0891-6934 print/1607-842X online
DOI: 10.3109/08916930903405867

informa
healthcare

Altered expression of the lymphocyte activation antigen CD30 in active celiac disease

N. PERIOLO[1], L. GUILLÉN[1], D. BERNARDO[2], S. I. NIVELONI[1], H. J. HWANG[3], J. A. GARROTE[3,4], J. C. BAI[3], E. ARRANZ[2], & A. C. CHERÑAVSKY[1]

[1]*División Inmunogenética, Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Buenos Aires, Argentina;* [2]*Mucosal Immunology Laboratory, Department of Pediatrics and Immunology, IBGM, Universidad de Valladolid-CSIC, Valladolid, Spain;* [3]*Sección Intestino Delgado, Departamento de Medicina, Hospital de Gastroenterología "Dr Carlos Bonorino Udaondo", Buenos Aires, Argentina and* [4]*Research Unit, Hospital Clínico Universitario, Valladolid, Spain*

(Received 10 June 2009; revised 7 October 2009; In final form 12 October 2009)

Abstract
Interleukin (IL)-15 and CD30 may be associated with the ongoing intestinal immunologic activation in celiac disease (CD). We studied duodenal biopsies and blood samples of patients with active CD (Cel) and controls in order to determine the regulatory role proposed for $CD30^+$ T cells in this Th1-driven disease and the potential influences of IL-15 on CD30 expression. We detected that a $CD30^+$ T-cell subpopulation persists longer in Cel after a 5 day incubation with anti-CD3 antibody than in controls ($p = 0.0063$). CD30 upregulation by IL-15 in T blasts was greater in Cel than in controls ($p = 0.0062$). At the mucosal compartment, the CD30 antigen was examined by immunohistochemistry and quantified on isolated lamina propria (LP) and epithelial T cells by flow cytometry. For Cel and controls, similar mean percentages of $CD3^+CD30^+$ intraepithelial T cells (5.88 vs. 5.51, p = ns) and LP T cells (7.38 vs. 7.49, p = ns) were observed at baseline and after *in vitro* gliadin challenge of duodenal biopsy samples. Our study demonstrates the occurrence of potentially important alterations of the immune response at the peripheral compartment. Our findings also allow us to speculate that a negative effect of soluble mediators at the mucosal compartment might counteract the latent influence of IL-15 on CD30 expression precluding a more severe course of active CD.

Keywords: *Active celiac disease, CD30 antigen, peripheral blood, small intestine mucosa, interleukin-15*

Abbreviations: *LT, Tlymphocytes; CD, celiac disease; Cel, patients with active celiac disease; HC, healthy controls; PBMC, peripheral blood mononuclear cells; LP, lamina propria; sCD30, soluble form of CD30; IEL, intraepithelial lymphocytes; HLA, human leukocyte antigens; LPL, lamina propria lymphocytes; EmA, anti-endomysial antibodies; tTG, anti-tissue transglutaminase; PBS, phosphate-buffered saline; HuTBl, human T lymphoblasts; HBSS, Hank's buffered salt solution*

Introduction

The CD30 glycoprotein has been initially recognized as expressed on Reed–Sternberg cells of Hodgkin's disease and other lymphoid malignancies [1,2]. Subsequent studies have identified that CD30 is an activation-induced antigen, mostly expressed in normal peripheral lymphoid organs, thymus, and diverse cellular lineages at variable extent [3]. CD30 and its surface-expressed ligand (CD30-L) are interacting glycoproteins belonging to the tumor necrosis factor (TNF)/nerve growth factor receptor and the TNF superfamilies, respectively. Their members control essential steps to the cell life cycle [4–6].

Exogenous inducers of CD30 include specific [7,8] or polyclonal (Phytohemaglutinnin (PHA) ConA, and anti-CD3) stimuli and co-stimulation via CD28 or recombinant cytokines like interleukin (IL)-12, IL-2, or IL-4 [9–11]. Although it is accepted that Th2/Th0 cell clones express CD30 *in vitro*, the actual role of $CD30^+$ T cells *in vivo* remains unclear since increased numbers of these cells were found associated either with Th2- or Th1- polarized diseases [12–15].

Correspondence: A. C. Cherñavsky, División Inmunogenética, Hospital de Clínicas "José de San Martín", Avenue Córdoba 2351, 3er Piso (CP1120), Capital Federal, Buenos Aires, Argentina. Tel: +54-011-59508756. Fax: +54-011-59508758. E-mail: accher@fibertel.com.ar.

The CD30 phenotype could be acquired through a process of activation and local differentiation at inflammatory sites. Alternatively, the origin of $CD30^+$ cells might be also the consequence of transmigration of previously activated T cells through endothelium. Independently from its hypothetical origin at inflamed sites, CD30 is an attractive activation marker to study at the duodenal mucosa by some reasons. First, the main requirement for $CD30^+$ T cell generation during local differentiation of T effectors is the activation of $CD45R0^+$ precursors that are already present at the duodenal compartment [16,17]. Second, specific mRNA has been detected in human peripheral blood T lymphocytes (LT) even in the absence of surface CD30 expression [18]. Thus, following the transmigration of activated T cells into the inflamed duodenal mucosa, a pre-activated condition (i.e. the presence of CD30-specific transcripts) might ensure CD30 antigen expression on T cell surface. Third, subsequently to $CD30^+$ cell *trans*-endothelial migration, local micro-environmental factors such as cytokines and/or antigens could further modulate CD30 expression.

Celiac disease (CD) is a Th1-dominated disorder characterized by the loss of immunologic tolerance to gluten in genetically susceptible human leukocyte antigen DQ2/-DQ8 individuals [19,20]. Activated lamina propria lymphocytes (LPL) generate cytokines leading to immune response and, ultimately, to villous atrophy. An increased number of intraepithelial lymphocytes (IEL) at the intestinal mucosal compartment, which are believed to exhibit cytotoxic and regulatory functions, are a hallmark of the disease [21].

A soluble form of CD30 (sCD30) produced by shedding from positive cells after proteolysis of an extracellular domain of the molecule [22] is associated with various diseases [23]. The correlation between serum sCD30 levels and the duodenal IEL infiltration has suggested that CD30 is indeed related to the ongoing intestinal immunologic activation of CD [24]. Besides these findings, many studies demonstrated an increased level of sCD30 and/or $CD30^+$ cells in a number of other pathologies associated with a predominant Th1 response that include infectious diseases and both systemic and organ-specific autoimmune disorders [25–28].

IL-15 is a chemotactic cytokine that seems to have a role in concert with adhesion molecules expressed on activated endothelia to direct extravasations to sites of inflammation [29]. At the duodenal mucosa, it is constitutively synthesized by many cell types including antigen-presenting, stromal, endothelial and epithelial cells. Among its pleiotropic effects, IL-15 induces the proliferation of both IEL [30] and LPL, the TCR-independent synthesis of interferon (IFN)-γ, and also upregulates CD25 expression on LPL [31]. This cytokine is likely produced in waves linked to gluten intake [32]. Although gliadin triggers an IL-15 mediated innate response in all individuals [33], the existence of a lower IL-15 immunological threshold may explain the origin of a secondary immune response developed in CD [34]. This distinctive secondary immune response generates villous atrophy [35,36] and a number of alterations on IEL, including the selective expansion of a $CD94^+$ subset [37], their conversion into lymphokine-activated killer cells [38], their high rate of proliferation, and enhanced IFN-γ/TNF-α secretion [39].

In refractory CD, a severe complication of the disorder defined as primary or secondary failure to respond to a gluten-free diet in CD, the uncontrolled overexpression of IL-15 induces the expansion and survival of typical abnormal lymphocytes [40]. Cell lines emerging from the culture of duodenal biopsies with IL-15 exhibit CD30 expression, in addition to the characteristic abnormal phenotype [41]. The CD30 phenotype is also associated with enteropathy-associated T-cell lymphoma (EATL) [42] and, in fact, CD30 expression by IEL in refractory CD may indicate a poor prognosis including the occurrence of overt lymphoma [43].

The reasons for the study of CD30 antigen at the duodenal mucosal, and also the demonstrated influences of IL-15 on mucosal T cells in the pathological context of CD and associated conditions (refractory CD, EATL), together with the regulatory role proposed for the presence of $CD30^+$ T cells in a different Th1-driven disease [44], prompted us to examine the role of $CD30^+$ T cells during active CD. To this end, we assessed the potential influences of IL-15 on CD30 expression at the peripheral compartment and examined the presence of $CD30^+$ cells in duodenal biopsies at baseline and after *in vitro* gliadin challenge.

Methods

Patients and control subjects

We assessed a total of 34 patients with active CD (Cel) and 38 unrelated healthy volunteers as controls (HC) who had biopsies from jejunum or distal duodenum taken during screening procedures for abdominal symptoms. All patients and control subjects were enrolled at the Small Intestinal Section of the "Dr C. B. Udaondo" Gastroenterology Hospital in Buenos Aires after they were informed of the aim of the study and gave their written, informed consent to be included during this procedure. The study was approved by the Ethics and Research Committees of the Gastroenterology Hospital informed of internationally endorsed standards for the application of the Helsinki Declaration.

Diagnosis of CD was based on the presence of clinical features, including a characteristic celiac enteropathy [45], increased duodenal IEL density

[46], positive anti-endomysial (EmA)/anti-tissue transglutaminase (tTG) antibodies [47], and DQ2 genotyping [48], as well as a response to a gluten-free diet. Moreover, autoimmune enteropathies were excluded by the absence of anti-enterocyte autoantibodies determined by indirect immunofluorescence using human small intestinal substrate [49]. All samples were obtained at the moment of diagnosis.

Monoclonal antibodies and reagents

Fluorochrome-conjugated mouse monoclonal (mAb) against human (hu) antigens: IgG1 peridinin–chlorophyll–protein complex (PerCP) anti-CD3 (clone SK7); IgG1 phycoerythrin (PE) anti-CD25 (clone 2A3); IgG1 fluorescein isothiocyanate (FITC) anti-CD103 (clone Ber-ACT8); and all isotype controls were obtained from Becton Dickinson (San José, CA, USA); mAb against human antigens: IgG2a PE anti-CD45RO (clone UCHL1) and IgG1 FITC anti-CD30 (clone BerH8) were obtained from Dako Corp. (Santa Barbara, CA, USA). For cell activation, mouse IgG1 mAb anti-CD3 (clone UCHT1) was obtained from Immunotech (Marseille, France). IgG1 anti-huIL-15 (clone 34505) and recombinant huIL-15 (rIL-15) were obtained from R&D Systems (Minneapolis, MN, USA). MOPC-21 (γ1, k) was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and served as a non-specific control.

Immunohistochemistry

On average, three serial sections of each tissue block were stained using a primary mAb anti-CD30: IgG_1, clone Ber-H2 (Dako) diluted 1:10 in phosphate-buffered saline (PBS) and incubated overnight at 4°C; negative control omitted the primary antibody. Sections were deparaffinized and rehydrated and endogenous peroxidase activity blocked (methanol–3% H_2O_2). Antigen retrieval was performed in a microwave oven by treatment of the sections in 10 mM sodium citrate buffer, pH 6.0, followed by a preincubation with horse serum (Vector Laboratories, Burlingame, CA, USA). A biotinylated antibody (Vectastain® Universal Elite, Vector Laboratories), horseradish peroxidase-conjugated ABC (Vectastain ABC KIT, Vector Laboratories), and 3,3′-diaminobenzidine substrate kit (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) were used. Sections were counterstained with 10% Harris's hematoxilin.

Cell preparations

Heparinized blood samples (10 ml) were obtained from each patient immediately after duodenal biopsy, and peripheral blood mononuclear cells (PBMC) were prepared by Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation, washed with RPMI 1640 medium (Life Technologies, Gaithersburg, MD, USA), and resuspended in medium supplemented with 10% heat-inactivated human AB serum, 2 mmol/l L-glutamine, and 50 μg/ml gentamicin (Sigma Chemical Co.).

Duodenal cells were dispersed from biopsy specimens as described previously [50]. Briefly, epithelial suspensions were obtained by incubating the tissue in 5 ml of Ca^{2+} and Mg^{2+}-free isotonic Hank's buffered salt solution (HBSS), pH 7.2, containing 1 mM EDTA and 1 mM dithiothreitol (Sigma) with moderate magnetic stirring for 1 h at 37°C and 200 rpm. The supernatant was harvested, and the cells were pelleted at 2000 rpm for 5 min and resuspended in isotonic PBS, pH 7.2 before immunostaining for CD30. This procedure yielded a median of 0.5×10^6 cells (range 0.4–1.0) with a median viability of 70% (range 55–85). Lamina propria (LP) suspensions were obtained by cutting the remainder of the tissue into small pieces followed by incubation with 1 mg/ml collagenase A (Sigma) in RPMI 1640, 2 mmol/l L-glutamine, and 50 μg/ml gentamicin. The mixture was subjected to moderate magnetic stirring for 2 h at 37°C and 200 rpm and yielded a median of 1.8×10^6 cells (range 0.4–3.1) with a median viability of 90% (range 75–95). After harvesting, the cells were washed in PBS and kept briefly on ice in RPMI 1640, 2 mmol/l L-glutamine, and 50 μg/ml gentamicin before immunostaining.

Immunostaining for duodenal cellular suspensions and non-stimulated PBMC

For staining of cell surface antigens, freshly isolated IEL-containing epithelial suspensions, LPL-containing LP suspensions, and PBMC were washed twice with 1 ml of PBS supplemented with 10% mouse serum for 10 min to block non-specific Fc binding and examined for CD30 expression at baseline. Disaggregated duodenal cells and PBMC were stained with CD30-FITC, CD3-PerCP, and CD25- or CD45RO-PE in triplicate. Isotype-matched negative control antibodies were used in all cases to assess background fluorescence intensity. Propidium iodide (Santa Cruz Biotechnology, CA, USA) at 0.5 μg/ml was added just before acquisition to exclude dead cells from the analysis of duodenal cell suspensions. A total of 15,000 and 30,000 $CD3^+$ events from duodenal cell suspensions and PBMC, respectively, were acquired. Acquisition and analysis were performed on a FACSCalibur flow cytometer (BD) using the WinMDI software version 2.8.

Gliadin challenge and mucosal tissue culture

Duodenal biopsy samples recollected from Cel and HC were washed and cultured *in vitro* for 3 h in

complete RPMI 1640 alone or with the addition of gliadin 0.1 mg/ml (Sigma). A duodenal basal explant culture in complete RPMI 1640 alone from each patient constituted an internal control of gliadin challenges. At the end of the incubation period, tissues were extensively washed with HBSS and duodenal cell suspensions were prepared and immunostained as mentioned previously.

Activation, DNA synthesis measures, and immunostaining of PBMC

For PBMC activation, cells was dispensed into 24-well microtiter plates (Costar, Cambridge, MA, USA), which were coated with 1 μg/ml anti-CD3 mAb at a density of 10^6 cells/ml of complete medium supplemented with 10% heat-inactivated huAB serum, and incubated for 2 h at 37°C in a humid atmosphere containing 5% CO_2. Cells were harvested 3 and 5 days later and either measured for CD30 expression by FC as described before (for assessment at baseline) or washed, pulsed with 1 μCi [^{3}H]-thymidine (Amersham, Aylesbury, UK) for the final 14 h of culture and harvested into glass-fiber filters. The incorporation of radioactivity was measured in a liquid scintillation counter.

Human T lymphoblasts (huTBl) obtained from Cel and controls by 3 day incubation with anti-CD3 as above, received further 3 day incubation with rIL-15 and were measured for CD30 expression at day 6. PBMC (10^6/ml) from other series of patients were cultured for 3 days in 24-well microtiter plates containing complete RPMI medium as above in the presence or absence of stimuli that included anti-CD3 (1μg/ml), anti-CD3 plus rIL-15 (50 ng/ml), anti-CD3 plus neutralizing anti-IL-15 antibody (10 ng/ml), or anti-CD3 plus MOPC-21, a non-specific control antibody (10 ng/ml). CD30 expression was measured by FC at day 3 as described previously.

Statistical analyses

The GraphPad Prism software (GraphPad, San Diego, CA, USA) was used for all analyses. The data were expressed as the mean value ± SEM of the number of analyzed samples. The statistical significance for differences between two groups was determined by a two-tailed Student's *t*-test as indicated. *p* values below 0.05 were considered as significant in all cases. Repeated measures ANOVA test with Bonferroni's correction for multiple comparisons was used to test for significant differences among three groups as indicated.

Results

The epidemiologic, laboratory, and histological information of the 34 Cel included in this study is shown in Table I. Ninety percent of the patients were genotyped as DQ2 positive. At diagnosis, all of them had typical histological characteristics and a classical clinical presentation of CD. Eighty percent of the patients presented anti-tTG and 20 of the 28 were also positive for EmA.

The surface CD30 induction on $CD3^+$ cells was assayed on PBMC after anti-CD3 stimulation. The expression of surface CD30 antigen was negligible on freshly isolated PBMC (day 0, before incubation with anti-CD3), but a discrete subpopulation of $CD3^+CD30^+$ cells was induced after the 3 day incubation with anti-CD3 in both experimental groups (Cel, 12.50 ± 10.00 and HC, 12.00 ± 9.00; p = ns). There was a marked difference in the kinetics of CD30 induction between Cel and HC (Figure 1, left panel). Meanwhile, CD30 expression declined by day 5 in HC, the $CD3^+CD30^+$ subpopulation remained increased in Cel (paired *t*-test analysis Cel day 3 vs. day 5, p = ns). By this time, a higher subset of $CD3^+CD30^+$ cells persists only in Cel (10.80 ± 2.35 vs. 1.40 ± 0.55, $p = 0.0063$). The lack of cell viability precluded the analysis of $CD3^+CD30^+$ subpopulations by day 7.

Anti-CD3 stimulation of PBMC also induced a lymphoproliferative response, as assayed by ^{3}H-thymidine incorporation, although no statistical differences were found in any case (Cel: 30,110 ± 1410 cpm and HC: 28,020 ± 1248 cpm, p = ns, Cel vs. HC at day 3; Cel: 3520 ± 700 cpm and HC: 2580 ± 405 cpm, p = ns, Cel vs. HC at day 5; data not shown).

The $CD30^+$ cells were identified specifically among $CD45RO^+CD25^+$ cells both in Cel and HC (Figure 1). The mean percentage ± SD for $CD30^+$ $CD45RO^+$ are 2.6 ± 2.3 (HC) and 11.3 ± 5.7 (Cel); both representative HC and Cel are shown in Figure 1(a,b). The mean percentage ± SD for $CD30^+CD25^+$ are 3.2 ± 1.2 (HC) and 10.3 ± 6.7 (Cel); both representative HC and Cel are shown in Figure 1(c,d). Although no triple staining for CD30, CD45R0, and CD25 in both PBMC and mucosal cells was performed, CD30 expression was found to have the same percentage of expression in both $CD3^+CD30^+CD45RO^+$ (Figure 1a,c) and $CD3^{+-}$ $CD30^+CD25^+$ (Figure 1b,d) staining either in Cel or in controls, strongly suggesting its expression on $CD3^+CD45RO^+CD25^+$ cells.

The human T blasts were obtained by incubation of PBMC with anti-CD3 mAb for 3 days (at 1×10^6 cells/ml) in RPMI 1640 supplemented with 10% human AB serum (more than 95% $CD3^+$ cells were obtained as determined by immunofluorescence; data not shown). As it was previously described, a similar CD30 expression on huTBl was observed for controls and Cel at day 3 (Figure 1). Blasts were treated during the following 3 days with a high rIL-15 concentration (50 ng/ml) that was able to induce its proliferative

Table I. Epidemiological, histological, and laboratory information of the Cel studied.

Case no.	Gender/age (year)	Marsh histological classification type	IELs/epithelial cells	tTG tests at diagnosis*	EmA tests at diagnosis†
1	F/55	III c	70	+	+
2	F/44	III c	52	−	+
3	F/38	III c	70	+	+
4	F/40	III c	34	+	+
5	M/25	III c	38.3	ND	+
6	F/39	III c	58.6	+	+
7	F/31	III c	39	+	+
8	F/30	III c	45	+	+
9	F/29	III c	32	+	+
10	F/45	III c	30	ND	+
11	F/19	III c	24	+	−
12	F/23	III c	36	+	+
13	F/35	III b	30.3	−	−
14	F/28	III b	28	+	ND
15	F/24	III a	39	+	+
16	F/55	III a	38	+	+
17	F/60	III a	30	+	+
18	F/69	III c	45	+	−
19	F/33	III c	44	+	ND
20	F/56	III c	ND	+	−
21	F/47	III c	ND	−	+
22	F/31	III c	40	+	−
23	F/37	III a	36	+	+
24	F/45	III c	40.4	+	+
25	M/43	III c	27	+	+
26	F/76	III c	40	+	+
27	M/56	III c	40	+	+
28	M/59	III c	41	+	ND
29	F/42	III a	35	+	−
30	M/54	III b	28	−	+
31	F/61	III c	63	+	+
32	F/67	III c	54	+	+
33	F/59	III c	52	+	+
34	M/33	III c	ND	+	+

CD, celiac disease; F, female; M, male; IgA, immunoglobulin A; IgG, immunoglobulin G; EmA, anti-endomysial antibodies; tTG, anti-tissue transglutaminase antibodies. *IgA and IgG subtypes of tTG were determined by ELISA using a commercial kit. Only the greater damage is reported as the description of the Marsh histological classification type; †IgA and IgG subtypes of EmA were determined by indirect immunofluorescence on monkey esophagus substrate [46].

activity [51], and CD30 surface expression was also measured by day 6 (Figure 2). The addition of rIL-15 to huTBl increased the relative numbers of $CD3^+CD30^+$ in both controls and Cel. HC and Cel were independently considered by the analysis of CD30 measures in huTBl + rIL-15 and huTBl, as paired data, for each patient (Figure 2(a),(b)). However, the $CD3^+CD30^+$ subset of LT upregulated by IL-15 was greater in Cel, as compared with HC by a two-tailed Student' *t*-test (Figure 2(c), mean value ± SEM are 19.55 ± 5.90 vs. 8.20 ± 1.50; $p = 0.025$).

To determine whether IL-15 is required for the development of the $CD30^+$ subset during the polyclonal anti-CD3-mediated activation of PBMC, we used a neutralizing anti-IL-15 mAb in four HC and four Cel (Figure 3).

In the graph belonging to one representative control, data report the mean ± SEM for values from three replicate cultures and are representative of four independent experiments. The stimulation with rIL-15 in RPMI 1640 did not induce detectable CD30 expression (not shown). Recombinant IL-15 showed an additive effect as judged by an increased percentage of $CD3^+CD30^+$ T cells (anti-CD3 + rIL-15 compared with anti-CD3 in RPMI 1640) as assayed by four independent proliferation challenge tests. However, we cannot discard the delivering of an additional co-stimulatory signal for cell proliferation of $CD3^+CD30^+$ T cells via CD30, given that CD30-L is spontaneously expressed by freshly isolated PBMC [52]. Stimulation of PBMC with anti-CD3 in the presence of anti-IL-15 mAb (anti-$CD3^+$ anti-IL-15) showed a reduction in the relative numbers of $CD3^+CD30^+$ T cells (3.00 ± 0.54) as compared with MOPC-21 control (11.00 ± 2.30), anti-CD3 (10.75 ± 1.90), and anti-CD3 plus rIL-15 (18.25 ± 5.60) mediated activation.

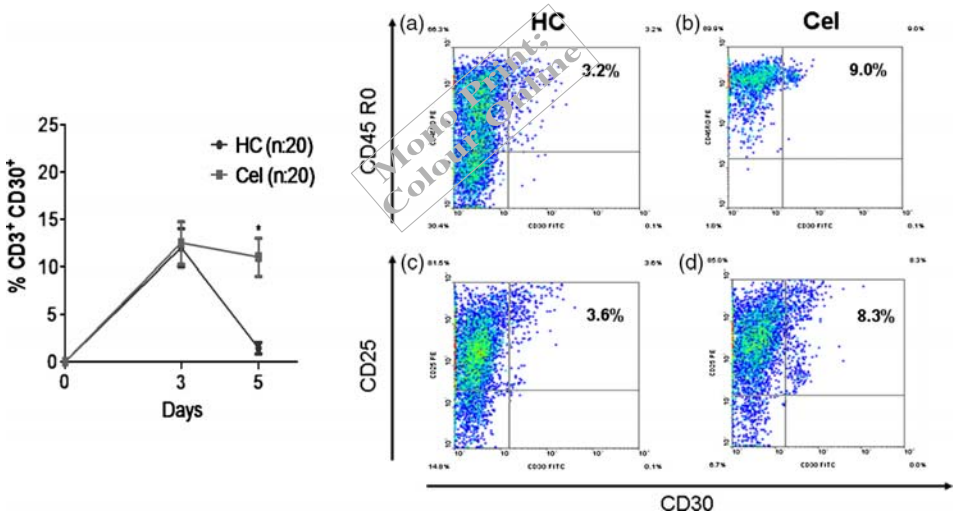

Figure 1. Left panel: PBMC were incubated with immobilized anti-CD3 and subsequently measured for CD30 expression after 3 and 5 days. The graphs show the analysis performed at day 5; similar results were obtained at day 3 (not shown). The percentages of $CD3^+CD30^+$ cells were measured by FC and the data were expressed as the mean ± SEM. *n*, number of patients; $\star p = 0.0063$, Cel vs. HC. The statistical significance for differences between the groups was determined by a two-tailed Student's *t*-test. Right panel: dot blots represent the expression of $CD30^+CD45RO^+$ (a and b) and $CD30^+CD25^+$ (c and d) T cells measured after a 5 day incubation with anti-CD3 for a representative HC (a and c) and Cel (b and d). Percentages of double positive cells are shown in the upper right corner of the respective quadrants. $CD30^+$ cells were examined on gated $CD3^+$ LT.

Given the fact that CD30 is expressed on a discrete subset of $CD45R0^+$ (Figure 1) and that a high percentage of both human duodenal LPL and IEL also express CD45R0 [16,17], we decided to examine the presence of $CD30^+$ cells at normal duodenum and in mucosal biopsies from Cel. The quantitative analysis of IEL and LPL suspensions is shown for a representative HC and Cel (Figure 4, upper panel). A >95% $CD3^+$ IEL was $CD103^+$, as determined by immunofluorescence (data not shown). Similar

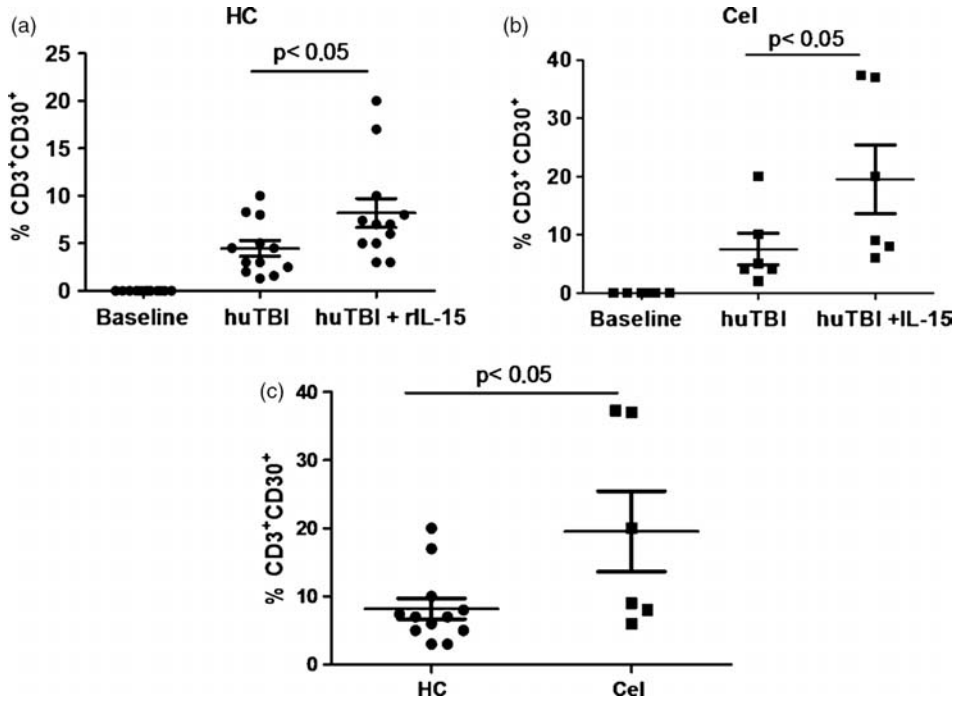

Figure 2. Shown are the compiled FC data displaying percentages of CD30 expression on $CD3^+$ gated lymphocytes at baseline, after a 3 day incubation with anti-CD3 (huTBl), and in huTBl further incubated for 3 days with rIL-15 (huTBl + rIL-15) in HC ($n = 10$) (a) and Cel ($n = 6$) (b) expressed as the mean ± SEM. A repeated measures ANOVA test with Bonferroni's correction for multiple comparisons was used to test for significant differences among groups. The percentages of $CD3^+CD30^+$ double positive huTBl after treatment with IL-15 were compared between HC and Cel in (c). The statistical significance for differences was determined by a two-tailed Student's *t*-test.

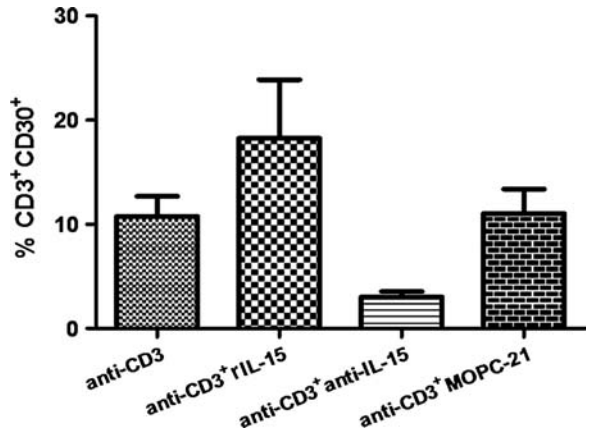

Figure 3. Peripheral blood mononuclear cells were activated with 1 μg/ml anti-CD3 for 3 days in all cases, in the presence of RPMI 1640 alone (anti-CD3), rIL-15 (anti-$CD3^+$rIL-15), neutralizing anti-IL-15 mAb (anti-$CD3^+$anti-IL-15), or MOPC-21 control mAb (anti-$CD3^+$MOPC-21). Data represent the mean ± SEM for three replicate cultures and are representative of four independent experiments. One control and one Cel were included in each experiment, and the graph belongs to a representative HC. The simultaneous co-stimulation with anti-CD3 and rIL-15 induced non-significant changes in the percentages of $CD3^+CD30^+$ double positive cells.

numbers of $CD30^+$ cells among $CD3^+$ T cells were observed in Cel and in controls, both in the epithelium (5.88 ± 1.62% vs. 5.51 ± 2.05%, p = ns, Cel vs. HC) and the LP (7.38 ± 1.51% vs. 7.49 ± 1.81%, p = ns, Cel vs. HC) respectively (Figure 4, middle panel). The immunohistochemical staining of biopsy samples revealed that $CD30^+$ cells are scattered in the mononuclear cell infiltrate of the LP and also in the epithelium, both in controls and Cel. The micro-photograph shows a representative control case (Figure 4, lower panel).

Biopsy samples were also cultured *in vitro* for 3 h in complete RPMI alone (baseline condition) or with the addition of gliadin, as described in Methods section. $CD3^+$ LPL and IEL were obtained at baseline and after gliadin challenge of cultures; CD30 expression was measured in paired samples (at baseline and after gliadin challenge) among HC and Cel, respectively. Gliadin challenge upregulated $CD30^+$ expression on $CD3^+$ cells in both LPL and IEL at the LP compartment. Upregulation was observed in three of the five samples from controls and in five of the six from Cel. However, no statistical differences were reached within both HC and Cel groups (Figure 5).

Gliadin stimulation indexes (SI) were determined by the ratio between percentage of $CD3^+CD30^+$ after *in vitro* culture of a piece of biopsy and percentage of $CD3^+CD30^+$ at baseline. SI ranged from 0.7 to 2.5 (in controls) and from 0.9 to 1.9 (in Cel). The comparison of SI performed by a two-tailed Student's *t*-test showed no statistical differences between the groups (data not shown). A similar lack of differences between controls and Cel was obtained for IEL (data not shown).

Discussion

The present study reveals that CD30 is as an activation marker following anti-CD3 stimulation on PBMC. Interestingly, we show that a $CD30^+$ subset from activated PBMC from Cel remained increased for a longer period of time than that observed in controls. The persistent expression of CD30 at day 5, when proliferation from the original activation begins to slow down, permits us to speculate that T blasts might be competent for *in vivo* CD30/CD30-L co-stimulatory interaction that, in turn, might regulate steps essential to the cell life cycle and/or the physiological Th1/Th2 balance. The upregulation of CD30 on mucosal lymphocyte subsets was suggested to be the source of the elevated serum levels of CD30 protein described in a number of autoimmune diseases [53]. However, due to the lack of detectable difference in mucosal $CD30^+$ cells neither *ex vivo* nor after gliadin challenge, its contribution as a source for the elevated sCD30 in serum that was previously described in CD [24] cannot be ensured from the present results.

Since the low and constitutive expression of CD30 in human T blasts from healthy volunteers was previously shown to be upregulated among surface markers during IL-15 stimulation [51], we used these cells as a model to evaluate the IL-15-driven effects on CD30 expression. With this aim, we investigated whether the presence of IL-15 was able to avoid the drop in $CD3^+CD30^+$ subpopulation observed after the 3 day incubation with anti-CD3. We demonstrated that the treatment of huTBl with rIL-15 not only impaired a drop in $CD3^+CD30^+$ subpopulations but also augmented a subpopulation of $CD30^+$ T blasts in both Cel and control individuals. Furthermore, there was an overall greater positive influence of rIL-15 on CD30 expression in Cel. We can speculate that our observations, though made at the peripheral compartment, are in line with the previously described overproduction of some innate mediators (i.e. nitrites and IFN-γ) after stimulation of biopsy explants from Cel. Those findings at the duodenal mucosa level suggested the existence of a lower IL-15 immunological threshold for the triggering of an inflammatory response in CD [34].

The use of a neutralizing anti-IL-15 mAb during anti-CD3 mitogenesis demonstrated a partial requirement for IL-15 in the generation of a $CD30^+$ subset. It appears to be of a similar magnitude, as judged by the percentages of double positive $CD3^+CD30^+$ cells, to the previously described need for IL-12 [9]. Also, the additive effect on the generation of $CD3^+CD30^+$ T cells during anti-CD3 mitogenesis in the presence of IL-15, is similar to the effect previously described for IL-12 by the same authors.

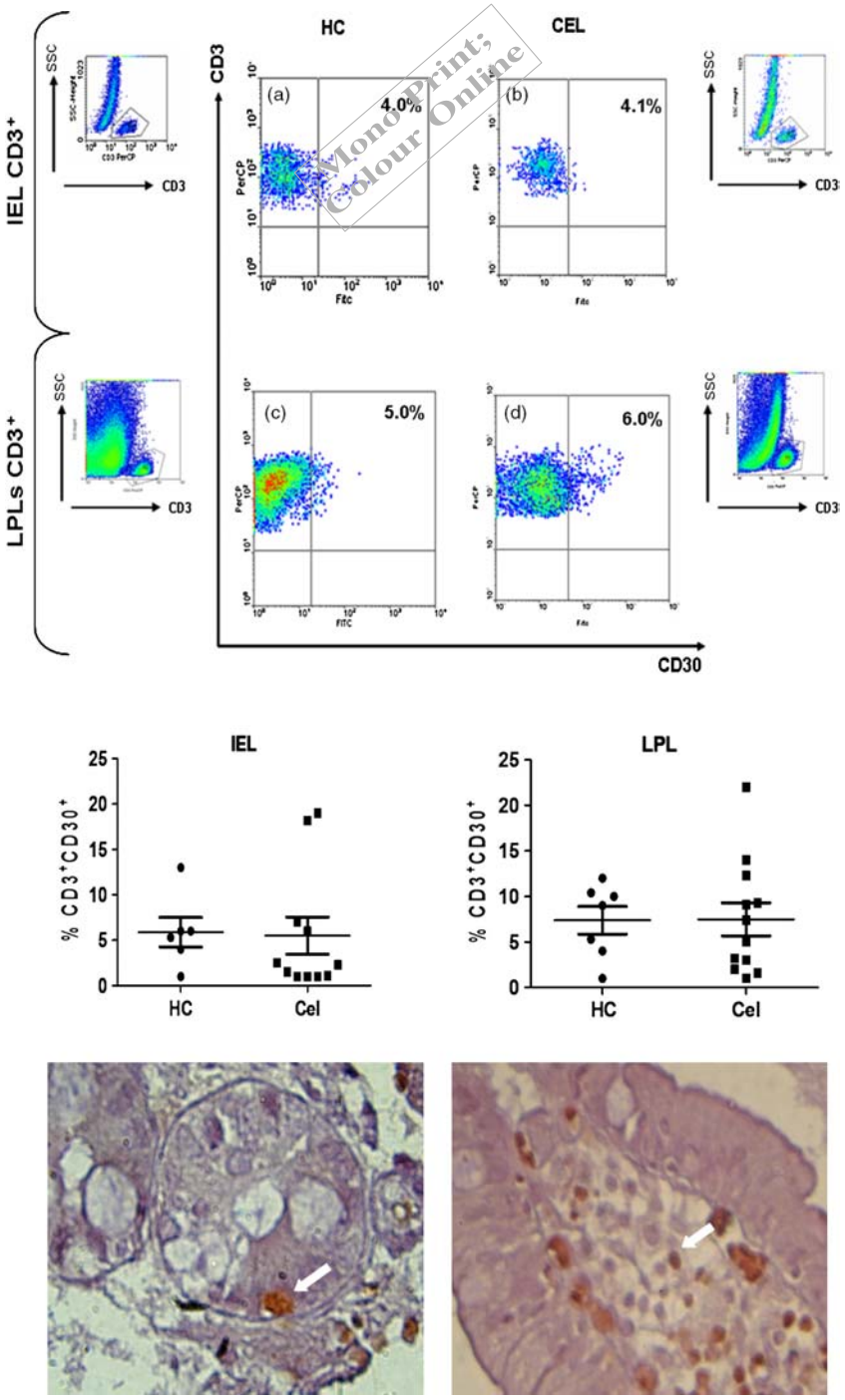

Figure 4. Based on side scatter and CD3 expression, a single gate was set on a selected lymphoid population defined as IEL $CD3^+$ cells and LPL $CD3^+$ cells. Upper panel: CD30 expression on viable $CD3^+$ cells in the lymphocyte region of duodenal epithelial (a and b) or LP suspensions (c and d) from one representative HC (a–c) and one Cel (b–d), respectively. Middle panel: the compiled FC data displaying CD30 expression on IEL and LPL, respectively, were expressed as the mean ± SEM. Comparisons among $CD3^+CD30^+$ subsets at the epithelium or the LP were not significant (Student's *t*-test). Lower panel: representative photomicrographs of immunostained paraffin-embedded sections of small intestinal tissue from a representative control. A CD30 positive IEL (left, original magnification 1000×) and a LPL (right, original magnification 400×) stained with 3,3′-diaminobenzidine are shown by the arrows.

Potentially important alterations of the immune response at the peripheral compartment were previously evidenced during active CD. Among others, changes in the pattern of cytokine production or in the signal transduction pathways within PBMC and alteration of peripheral regulatory functions were described [54–57]. Our results also point toward the occurrence of peripheral manifestations of the disease.

The higher influence of rIL-15 on CD30 expression at the peripheral compartment in active CD and the previously demonstrated local production of IL-15 after gluten intake raised the possibility that $CD30^+$

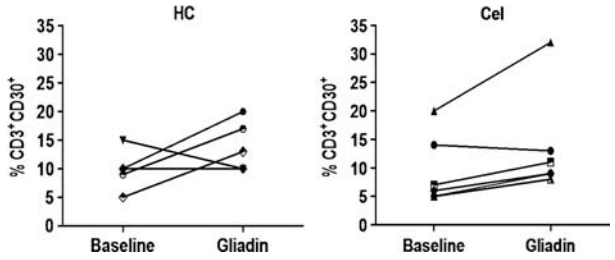

Figure 5. Biopsy samples from HC ($n = 5$) and Cel ($n = 6$) were cultured *in vitro* for 3 h in RPMI 1640 alone (baseline) or with the addition of gliadin (0.1 mg/ml). As indicated, the graphs represent the paired results for HC and Cel groups.

mucosal cells could also be involved in the IL-15-driven immune response to gluten at the local compartment. Our results failed to demonstrate any difference between both groups either at baseline conditions or the generation of $CD3^+CD30^+$ LPL (IEL, not shown) after duodenal gliadin stimulation. Even though the direct effect of rIL-15 on CD30 expression at the local compartment (either on isolated T cells or on biopsy explants) remains to be evaluated, our results can be interpreted as follows.

The similar CD30 expression observed at baseline conditions allows us to hypothesize that the generation of $CD3^+CD30^+$ mucosal T cells might be involved in the general IL-15-driven innate response to gluten, previously described in all individuals. In this scenario, IL-15 might act as a chemoattractant cytokine and collaborate with adhesion molecules for the activation (and CD30 expression) of peripheral blood T cells recruited into the duodenal mucosa [29,58]. Subsequently, the duodenal cytokine environment might play a key role in $CD30^+$ T cell survival.

The similar generation of $CD3^+CD30^+$ LPL after gliadin stimulation of biopsy explants allows us to hypothesize that large amounts of cytokines such as IL-10 and IFN-γ [59], two known negative regulators for CD30 expression [9,11], might counteract the IL-15-driven effects on CD30 expression. Since increased numbers of either $CD30^+$ IEL or LPL might worsen the disease through CD30 survival signals and clonal IEL proliferation, or by the massive production of mediators of tissue injury (i.e. IFN-γ) by LPL, a putative homeostatic balance acting on this molecule might preclude a more severe course of the active disease. In fact, it was previously demonstrated that either the interplay between local factors, such as the impairment of TGF-β/Smads signaling by IL-15 [60] or the inappropriate balance between IL-10 and IFN-γ [59] is involved in the breakdown of the intestinal homeostasis.

Both the differential kinetics of CD30 expression and the distinctive IL-15 regulation of CD30 antigen on PBMC highlight the occurrence of peripheral manifestations of CD. In addition, our observations at the local compartment point toward the existence of an interplay between regulators of CD30 expression (i.e. IL-10, IFN-γ, and IL-15), which might prevent the increase of $CD3^+CD30^+$ mucosal T cell generation during the active disease. Also, the herein described influences of IL-15 on CD30 antigen at the peripheral compartment, together with the suggested regulatory role of local micro-environmental factors on CD30 expression, deserve to be further investigated during severe complications of CD that are clearly associated with a high production of IL-15.

Acknowledgements

We wish to thank the patients and their families for their cooperation and blood samples.

Declaration of interest: This study was funded by the Buenos Aires University (M010), CONICET (PIP 6104), ANPCyT (06-257), and Fundación Carolina and Fundación Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL). The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Schwab U, Stein H, Gerdes J, Lemke H, Kirchner H, Schaadt M, Diehl V. Production of a monoclonal antibody specific for Hodgkin and Sternberg–Reed cells of Hodgkin's disease and a subset of normal lymphoid cells. Nature 1982;299:65–67.

[2] Stein H, Mason DY, Gerdes J, O'Conner N, Wainscoat J, Pallsen G, Gatter K, Falini B, Delsol G, Lemke H. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue. Evidence that Reed Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 1985;66:848–858.

[3] Chiarle R, Podda A, Prolla G, Gong J, Thorbecke GJ, Inghirami G. CD30 in normal and neoplastic cells. Clin Immunol 1999;90:157–164.

[4] Durkop H, Latza U, Hummel M, Eitelbach F, Seed B, Stein H. Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell 1992;68:421–427.

[5] Smith CA, Gruss HJ, Davis T, Anderson D, Farrah T, Baker E, Sutherland GR, Brannan CI, Copeland NG, Jenkins NA, Grabstein KH, Gliniak B, McAlister IB, Fanslow W, Alderson M, Falk B, Gimpel S, Gillis S, Din WS, Goodwin RG, Armitage RJ. CD30 antigen, a marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 1993;73:1349–1360.

[6] Smith CA, Farrah T, Goodwin RG. The TNF receptor superfamily of cellular and viral proteins: Activation, co-stimulation, and death. Cell 1994;76:959–962.

[7] Tarkowski M, Chrul S, Bodalski J. The effect of IL-18 on IL-12-induced CD30 expression and IL-4 and IFN-g production by allergen and PPD specific T cells. Clin Exp Immunol 2002;127:78–84.

[8] Munk ME, Kern P, Kaufmann SH. Human $CD30^+$ cells are induced by *Mycobacterium tuberculosis* and present in tuberculosis lesions. Int Immunol 1997;9:713–720.

[9] Alzona M, Jack HM, Fisher RI, Ellis TM. IL-12 activates IFN-y production through the preferential activation of CD30+ T cells. J Immunol 1995;154:9–16.
[10] Ellis TM, Simms PE, Slivnick DJ, Jack HM, Fisher RI. CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO$^+$T cells. J Immunol 1993; 151:2380–2389.
[11] Nakamura T, Lee RK, Nam SY, Al-Ramadi BK, Koni PA, Bottomly KJ. Reciprocal regulation of CD30 expression on CD4$^+$T cells by IL-4 and IFN-gamma. J Immunol 1997; 158:2090–2098.
[12] Chilosi M, Facchetti F, Notarangelo LD, Romagnani S, Del Prete G, Almerigogna F, De Carli M, Pizzolo G. CD30 cell expression and abnormal soluble CD30 serum accumulation in Omenn's syndrome: Evidence for a T helper 2-mediated condition. Eur J Immunol 1996;26:329–334.
[13] Piletta PA, Wirth S, Hommel L, Saurat JH, Hauser C. Circulating skin-homing T cells in atopic dermatitis. Selective upregulation of HLA-DR, interleukin-2R, and CD30 and decrease after combined UV-A and UV-B phototherapy. Arch Dermatol 1996;132:1230–1232.
[14] Munk ME, Kern P, Kaufmann SH. Human CD30 + cells are induced by *Mycobacterium tuberculosis* and present in tuberculosis lesions. Int Immunol 1997;9:713–720.
[15] Krams SM, Cao S, Hayashi M, Villanueva JC, Martinez OM. Elevations in IFN-gamma, IL-5, and IL-10 in patients with the autoimmune disease primary biliary cirrhosis: Association with autoantibodies and soluble CD30. Clin Immunol Immunopathol 1996;80:311–320.
[16] Schieferdecker HL, Ullrich R, Hirseland H, Zeitz M. T cell differentiation antigens on lymphocytes in the human intestinal lamina propria. J Immunol 1992;149:2816–2822.
[17] Eiras P, Roldan E, Camarero C, Olivares F, Bootello A, Roy G. Flow cytometry description of a novel CD3/CD7 intraepithelial lymphocyte subset in human duodenal biopsies: Potential diagnostic value in coeliac disease. Cytometry 1998;34: 95–102.
[18] Gerli R, Pitzalis C, Bistoni O, Falini B, Costantini V, Russano A, Lunardi C. CD30+ T cells in rheumatoid synovitis: Mechanisms of recruitment and functional role. J Immunol 2000;164:4399–4407.
[19] Trier JS. Celiac sprue. N Engl J Med 1991;325:1709.
[20] van Heel DA, Hunt K, Greco L, Wijmenga C. Genetics in coeliac disease. Best Pract Res Clin Gastroenterol 2005;19: 323–339.
[21] Sollid LM. Intraepithelial lymphocytes in celiac disease: License to kill revealed. Immunity 2004;21:303–304.
[22] Hansen HP, Kisselva T, Kobarg J, Horn-Lohrens O, Havsteen B, Lemke H. A zinc metalloproteinase is responsible for the release of CD30 on human tumor cell lines. Int J Cancer 1995; 63:750–756.
[23] Horie R, Watanabe T. CD30: Expression and function in health and disease. Semin Immunol 1998;10:457–470.
[24] Augustin MT, Kokkonen J, Karttunen R, Karttunen TJ. Serum granzymes and CD30 are increased in children's milk protein sensitive enteropathy and celiac disease. J Allergy Clin Immunol 2005;115:157–162.
[25] Munk ME, Kern P, Kaufmann SH. Human CD30+ cells are induced by *Mycobacterium tuberculosis* and present in tuberculosis lesions. Int Immunol 1997;9:713–720.
[26] Okumura M, Hidaka Y, Kuroda S, Takeoka K, Tada H, Amino N. Increased serum concentrations of soluble CD30 in patients with Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab 1997;82:1757–1760.
[27] Krams SM, Cao S, Hayashi M, Villanueva JC, Martinez OM. Elevations in IFN-γ, IL-5, and IL-10 in patients with the autoimmune disease primary biliary cirrhosis: Association with autoantibodies and soluble CD30. Clin Immunol Immunopathol 1996;80:311–320.
[28] Gerli R, Muscat C, Bistoni O, Falini B, Tomassini C, Agea E, Tognellini R, Biagini P, Bertotto A. High levels of the soluble form of CD30 molecule in rheumatoid arthritis (RA) are expression of CD30$^+$T cell involvement in the inflamed joints. Clin Exp Immunol 1995;102:547–550.
[29] Sancho D, Yáñez-Mó M, Tejedor R, Sánchez-Madrid F. Activation of peripheral blood T cells by interaction and migration through endothelium: Role of lymphocyte function antigen-1/intercellular adhesion molecule-1 and interleukin-15. Blood 1999;93:886–896.
[30] Ebert EC. Interleukin 15 is a potent stimulant of intraepithelial lymphocytes. Gastroenterology 1998;115:1439–1445.
[31] Ebert EC, Jabri B. Massive interleukin-12-induced interferon-γ production by interleukin-15-stimulated lamina propria lymphocytes followed by down-regulation of the interleukin-12 receptor. Immunology 2008;124:453–460.
[32] León AJ, Garrote JA, Blanco-Quirós A, Calvo C, Fernández-Salazar L, Del Villar A, Barrera A, Arranz E. Interleukin 18 maintains a long-standing inflammation in coeliac disease patients. Clin Exp Immunol 2006;146:479–485.
[33] Bernardo D, Garrote JA, Fernández-Salazar L, Riestra S, Arranz E. Is gliadin really safe for non-coeliac individuals? Production of interleukin 15 in biopsy culture from non-coeliac individuals challenged with gliadin peptides. Gut 2007; 56:889–890.
[34] Bernardo D, Garrote JA, Allegretti Y, León A, Gómez E, Bermejo-Martin JF, Calvo C, Riestra S, Fernández-Salazar L, Blanco-Quirós A, Chirdo F, Arranz E. Higher constitutive IL15R alpha expression and lower IL-15 response threshold in coeliac disease patients. Clin Exp Immunol 2008;154:64–73.
[35] Maiuri L, Ciacci C, Auricchio S, Brown V, Quaratino S, Londei M. Interleukin 15 mediates epithelial changes in celiac disease. Gastroenterology 2000;119:996–1006.
[36] Hüe S, Mention JJ, Monteiro RC, Zhang S, Cellier C. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. Immunity 2004;21:367–377.
[37] Jabri B, de Serre NP, Cellier C, Evans K, Gache C, Carvalho C, Mougenot JF, Allez M, Jian R, Desreumaux P, Colombel JF, Matuchansky C, Cugnenc H, Lopez-Botet M, Vivier E, Moretta A, Roberts AI, Ebert EC, Guy-Grand D, Brousse N, Schmitz J, Cerf-Bensussan N. Selective expansion of intraepithelial lymphocytes expressing the HLA-E-specific natural killer receptor CD94 in celiac disease. Gastroenterology 2000;118:867–879.
[38] Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity 2004;21:357–366.
[39] Di Sabatino A, Ciccocioppo R, Cupelli F, Cinque B, Millimaggi D, Clarkson MM. Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease. Gut 2006;55:469–477.
[40] Mention JJ, Ben Ahmed M, Bègue B, Barbe U, Verkarre V, Asnafi V, Colombel JF, Cugnenc PH, Ruemmele FM, McIntyre E, Brousse N, Cellier C, Cerf-Bensussan N. Interleukin 15: A key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology 2003;125:730–745.
[41] Tjon JM, Verbeek WH, Kooy-Winkelaar YM, Nguyen BH, van der Slik AR, Thompson A, Heemskerk MH, Schreurs MW, Dekking LH, Mulder CJ, van Bergen J, Koning F. Defective synthesis or association of T-cell receptor chains underlies loss of surface T-cell receptor-CD3 expression in enteropathy-associated T-cell lymphoma. Blood 2008;112: 5103–5110.

[42] Murray A, Cuevas D, Jones DB, Wright DH. Study of the immunohistochemistry and T cell clonality of enteropathy-associated T cell lymphoma. Amer J Pathol 1995;146: 509–519.

[43] Farstad IN, Johansen FE, Vlatkovic L, Jahnsen J, Scott H, Fausa O, Bjørneklett A, Brandtzaeg P, Halstensen TS. Heterogeneity of intraepithelial lymphocytes in refractory sprue: Potential implications of CD30 expression. Gut 2002; 51:372–378.

[44] Gerli R, Lunardi C, Vinante F, Bistoni O, Pizzolo G, Pitzalis C. Role of $CD30^+$ T cells in rheumatoid arthritis: A counter regulatory paradigm for Th1-driven diseases. Trends Immunol 2001;22:72–77.

[45] Marsh MM. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity (celiac sprue). Gastroenterology 1992;102:330–354.

[46] Cabanne A, Vázquez H, Argonz J, Moreno ML, Nachman F, Niveloni S, Mazure R, Kogan Z, Gómez JC, Mauriño E, Bai JC. Clinical utility of counting intraepithelial lymphocytes in celiac disease intestinal mucosa. Acta Gastroenterol Latinoam 2007;37:20–28.

[47] Sugai E, Selvaggio G, Vazquez H, Viola M, Mazure R, Pizarro B, Smecuol E, Flores D, Pedreira S, Mauriño E, Gomez JC, Bai JC. Tissue transglutaminase in celiac disease: Assessment of a commercial kit. Am J Gastroenterol 1997;95:2318–2322.

[48] Olerup O, Alder A, Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in two hours. Tissue Antigens 1993;41:119–134.

[49] Corazza GR, Biagi F, Volta U, Andreani ML, De Franceschi L, Gasbarrini G. Autoimmune enteropathy and villous atrophy in adults. Lancet 1997;350:359–360.

[50] Madrigal L, Lynch S, Feighery C, Weir D, Kelleher D, O'Farrelly CJ. Flow cytometry analysis of surface major histocompatibility complex class II expression on human epithelial cells prepared from small intestinal biopsies. Immunol Meth 1993;158:207–214.

[51] Bulfone-Paus S, Dürkop H, Paus R, Krause H, Pohl T, Onu A. Differential regulation of human T lymphoblast functions by IL-2 and IL-15. Cytokine 1997;9:507–513.

[52] Kilwinski J, Berger T, Mpalaskas J, Reuter S, Flick W, Kern P. Expression of CD30 mRNA, CD30L mRNA and a variant form of CD30 mRNA in restimulated peripheral blood mononuclear cells (PBMC) of patients with helminthic infections resembling a Th2 disease. Clin Exp Immunol 1999;115:114–119.

[53] Thomas PD, Forbes A, Nicholls RJ, Ciclitira PJ. Altered expression of the lymphocyte activation markers CD30 and CD27 in patients with pouchitis. Scand J Gastroenterol 2001; 36:258–264.

[54] O'Keeffe J, Mills K, Jackson J, Feighery C. T cell proliferation, MHC class II restriction and cytokine products of gliadin stimulated peripheral blood mononuclear cells (PBMC). Clin Exp Immunol 1999;117:269–276.

[55] Mizrachi A, Broide E, Buchs A, Kornberg A, Aharoni D, Bistritzer T, Rapoport MJ. Lack of correlation between disease activity and decreased stimulated secretion of IL-10 in lymphocytes from patients with celiac disease. Scand J Gastroenterol 2002;37:924–930.

[56] Broide E, Scapa E, Bloch O, Shapiro M, Kimchi NA, Ben-Yehudah G, Rapoport MJ. Evidence for aberrant regulation of MAP kinase signal transduction pathway in peripheral blood mononuclear cells in patients with active celiac disease. Dig Dis Sci 2009;54:1270–1275.

[57] Granzotto M, Dal Bo S, Quaglia S, Tommasini A, Piscianz E, Valencic E, Ferrara F, Martelossi S, Ventura A, Not T. Regulatory T-cell function is impaired in celiac disease. Dig Dis Sci 2008;54(7):1513–1519.

[58] Gerli R, Pitzalis C, Bistoni O, Falini B, Costantini V, Russano A, Lunardi C. CD30 T cells in rheumatoid synovitis: Mechanisms of recruitment and functional role. J Immunol 2000;164:4399–4407.

[59] Gianfrani C, Levings MK, Sartirana C, Mazzarella G, Barba G, Zanzi D, Camarca A, Iaquinto G, Giardullo N, Auricchio S, Troncone R, Roncarolo MG. Gliadin-specific type 1 regulatory T cells from the intestinal mucosa of treated celiac patients inhibit pathogenic T cells. J Immunol 2006;177: 4178–4186.

[60] Benahmed M, Meresse B, Arnulf B, Barbe U, Mention JJ, Verkarre V, Allez M, Cellier C, Hermine O, Cerf-Bensussan N. Inhibition of TGF-beta signaling by IL-15: A new role for IL-15 in the loss of immune homeostasis in celiac disease. Gastroenterology 2007;132:994–1008.