Potential role of the IL-33/ST2 axis in celiac disease
The IL-33/ST2 axis has been implicated in the pathogenesis of several tissue-specific autoimmune diseases. Celiac disease (CD) is the only autoimmune disease in which both the major genetic factors (HLA-DQ2/DQ8) and etiologic ones (dietary gluten) for susceptibility are known. We have measured serum levels and determined intestinal tissue expression of IL-33 and its receptor soluble ST2 in patients with CD to investigate their association with disease activity. Serum and tissue levels of both IL-33 and sST2 were significantly higher in patients with CD compared with those in control patients without CD. We show that toxic peptides extracted from barley and wheat gliadin significantly stimulate the production of IL-33 and ST2 in cultured peripheral blood mononuclear cell from celiac patients, strongly implicating the IL-33/ST2 axis in the pathogenesis of CD. The higher levels of IL-33 and its receptor ST2 in tissue and serum reflect an active inflammatory state and may represent a potential biomarker for disease activity. A better understanding of IL-33/ST2 release, mode of action, and regulation will be crucial to develop therapeutics that target the IL-33/ST2 pathway to treat CD.

INTRODUCTION

Celiac disease (CD) is an autoimmune disorder with genetic, environmental, and immunological factors governing sensitivity to gluten protein in food. The prevalence of CD in Western countries is about 1 in 250 people, but some estimates go as high as 1% of the population.1-3

Gluten ingestion by patients with CD leads to a cascade of inflammatory reactions and eventually to the hallmark small-intestinal lesion, the most important consequence of which is diminished nutrient uptake, characterized by CD4+ T-cell activation, increasing numbers of intraepithelial lymphocytes with partial to total villus atrophy.2,4-6 A common feature of gluten-derived epitopes is the presence of multiple proline and glutamine residues that are selectively deamidated by tissue transglutaminase. The modified peptides are able to bind to HLA-DQ2/DQ8, stimulating CD4+ T-helper 1 (Th1) cells in the lamina propria. These T cells become activated upon recognition of gluten peptides and produce many different cytokines, predominantly interferon gamma (IFN-γ), resulting in an inflammatory response in the small intestine that leads to a flattening of the mucosa.7-8

The 33-mer peptide is one of the most highly antigenic peptides identified to date.9 This peptide was identified as the primary initiator of the inflammatory response to gluten in CD patients. Sequence alignment has shown that homologues of the 33-mer peptide are found in wheat gliadins, barley hordeins, and rye secalins, all of which are toxic cereals in the CD diet.10-11

In a previous work, we have investigated the identification of peptides in barley and wheat gliadin varieties with a reduced or increased immunotoxicity profile for the celiac patients, assessing the affinity of the anti-gliadin 33-mer G12 moAb against different lines of barley and wheat in order to identify the potential toxicity of each one. We demonstrated a direct correlation of the reactivity with G12 moAb and the immunogenicity that was observed by measuring cell proliferation and IFN-γ release as indexes of peripheral blood mononuclear cell (PBMC) activation.10-12

Interleukin-33 is a member of the IL-1 family of cytokines that has recently emerged as a key regulator of inflammatory and immune processes.13-16 It is also a chromatin-associated protein acting as a transcriptional regulator, making it a dual-function protein that is both a cytokine and a nuclear factor.17 IL-33 is expressed primarily in non-hematopoietic cells including fibroblasts, epithelial cells, and endothelial cells but is also present in cells of hematopoietic origin, particularly in...
macrophages and dendritic cells (DCs). IL-33 is considered to be involved in a broad spectrum of diseases: not only in Th2-related diseases such as asthma and atopic dermatitis, but also in other inflammatory diseases such as inflammatory bowel disease, type-2 diabetes, and cardiovascular diseases. IL-33 regulates innate and acquired immunity through binding to the membrane-bound ST2 molecule (ST2L) of the IL-33R complex expressed in human Th2 cells, mast cells, natural killer (NK) cells, myeloid cells and DCs. Once in the extracellular environment, IL-33 has been proposed to act as a danger-associated molecular pattern or alarmin.

ST2 is the receptor for IL-33 and a member of the IL-1 receptor family. There are at least three isoforms of ST2 gene production, resulting from different splicing of the gene, a soluble form of ST2 (sST2), a membrane-bound form (ST2L), and a constitutively active variant ST2. ST2L functions as a transmembrane signaling receptor for IL-33 by mediating the effect of IL-33 in the inflammatory process. Soluble ST2, a receptor that can suppress IL-33 activity, acts as a decoy receptor for IL-33 and functionally inhibits IL-33 activity in vitro as well as in vivo. In several disorders, serum concentrations of sST2 were high, and the binding ability of sST2 toward IL-33 is generally thought to be key in the pathogenetic relevance of sST2.

In this study, we evaluate the role of the IL-33/ST2 system in innate immunity of the intestinal mucosa and its importance in CD.

**MATERIAL AND METHODS**

**Patients**

A total of 20 patients with biopsy-proven active CD and 15 control patients in whom CD was ruled out were included in the control non-celiac group formed by patients who had no history of immune-mediated diseases (n = 8) and active Crohn’s disease patients (n = 7). The control was taken among patients with negative serum anti-TG2 and endomysial antibodies that have received a different diagnostic from CD. No patient with refractory CD was included into the study. The Ethics Committee of Virgen de las Nieves Hospital, Granada (Spain) approved the study, and informed consent was obtained from the participants. The characteristics of the CD patients are summarized in Table 1.

**Serological and histological analysis**

The diagnosis of CD was established in the patients by serological screening tests, using anti-endomysial antibodies (AEMA), anti-tissue transglutaminase antibodies, accompanied by a biopsy of the small intestine and specific HLA typing (HLA-DQ/DR). Biopsy specimens of small intestine in CD patients were taken by gastrointestinal endoscopy. The small-intestine biopsy specimens were classified according to the criteria of Marsh with modifications. Similarly, at the time of collecting the samples, in order to confirm the CD diagnosis, we also collected peripheral blood, and a small-intestinal mucosa biopsy.

**Immunohistochemical staining**

Formalin-fixed paraffin-embedded biopsies from patients with active CD and control subjects were used. About 4 μm thick sections were cut, deparaffinized, and rehydrated. We used anti-IL-33 antibody (Abcam, Cambridge, UK) and anti-ST2 antibody (Abcam, Cambridge, UK). Immunohistochemical staining was performed using UltraTech HRP.

### Table 1. Clinical data of celiac patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>ATGA (IgA)</th>
<th>AEMA</th>
<th>Atrophy grade (marsh criteria)</th>
<th>HLA-DQB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac 1</td>
<td>4</td>
<td>Female</td>
<td>20</td>
<td>106</td>
<td>&gt;200</td>
<td>+</td>
<td>IV</td>
<td>0201-0202</td>
</tr>
<tr>
<td>Celiac 2</td>
<td>4</td>
<td>Female</td>
<td>17.5</td>
<td>108</td>
<td>&gt;200</td>
<td>+</td>
<td>III C</td>
<td>0201-0202</td>
</tr>
<tr>
<td>Celiac 3</td>
<td>1</td>
<td>Female</td>
<td>7.5</td>
<td>76</td>
<td>&gt;150</td>
<td>+</td>
<td>III B</td>
<td>0301-0302</td>
</tr>
<tr>
<td>Celiac 4</td>
<td>3</td>
<td>Female</td>
<td>11</td>
<td>90</td>
<td>&gt;50</td>
<td>+</td>
<td>III A</td>
<td>0201-0603</td>
</tr>
<tr>
<td>Celiac 5</td>
<td>12</td>
<td>Male</td>
<td>49</td>
<td>151</td>
<td>&gt;200</td>
<td>+</td>
<td>IV</td>
<td>0201-0503</td>
</tr>
<tr>
<td>Celiac 6</td>
<td>7</td>
<td>Male</td>
<td>23</td>
<td>123</td>
<td>&gt;125</td>
<td>+</td>
<td>III A</td>
<td>0201-0301</td>
</tr>
<tr>
<td>Celiac 7</td>
<td>1</td>
<td>Male</td>
<td>10.5</td>
<td>82</td>
<td>&gt;125</td>
<td>+</td>
<td>II</td>
<td>0201-0602</td>
</tr>
<tr>
<td>Celiac 8</td>
<td>5</td>
<td>Female</td>
<td>19</td>
<td>108</td>
<td>&gt;125</td>
<td>+</td>
<td>III A</td>
<td>0201-0501</td>
</tr>
<tr>
<td>Celiac 9</td>
<td>10</td>
<td>Female</td>
<td>23.5</td>
<td>129</td>
<td>&gt;200</td>
<td>+</td>
<td>IV</td>
<td>0201-0301</td>
</tr>
<tr>
<td>Celiac 10</td>
<td>2</td>
<td>Female</td>
<td>14</td>
<td>93</td>
<td>&gt;125</td>
<td>+</td>
<td>III B</td>
<td>0301-0302</td>
</tr>
<tr>
<td>Celiac 11</td>
<td>10</td>
<td>Female</td>
<td>24</td>
<td>132</td>
<td>&gt;125</td>
<td>+</td>
<td>III B</td>
<td>0201-0202</td>
</tr>
<tr>
<td>Celiac 12</td>
<td>2</td>
<td>Female</td>
<td>13</td>
<td>92</td>
<td>15</td>
<td>+</td>
<td>III A</td>
<td>0301-0302</td>
</tr>
<tr>
<td>Celiac 13</td>
<td>3</td>
<td>Male</td>
<td>13.5</td>
<td>91</td>
<td>&gt;90</td>
<td>+</td>
<td>III C</td>
<td>0201-0604</td>
</tr>
<tr>
<td>Celiac 14</td>
<td>5</td>
<td>Male</td>
<td>17.5</td>
<td>106</td>
<td>&gt;200</td>
<td>+</td>
<td>III C</td>
<td>0201-0202</td>
</tr>
<tr>
<td>Celiac 15</td>
<td>6</td>
<td>Female</td>
<td>18.5</td>
<td>nd</td>
<td>&gt;150</td>
<td>+</td>
<td>III A</td>
<td>0201-0501</td>
</tr>
<tr>
<td>Celiac 16</td>
<td>8</td>
<td>Female</td>
<td>23.5</td>
<td>127</td>
<td>&gt;125</td>
<td>+</td>
<td>III A</td>
<td>0201-0503</td>
</tr>
<tr>
<td>Celiac 17</td>
<td>2</td>
<td>Male</td>
<td>10</td>
<td>91</td>
<td>&gt;125</td>
<td>+</td>
<td>III B</td>
<td>0201-0301</td>
</tr>
<tr>
<td>Celiac 18</td>
<td>11</td>
<td>Female</td>
<td>25.5</td>
<td>135</td>
<td>&gt;200</td>
<td>+</td>
<td>IV</td>
<td>0201-0202</td>
</tr>
<tr>
<td>Celiac 19</td>
<td>7</td>
<td>Male</td>
<td>21</td>
<td>125</td>
<td>&gt;200</td>
<td>+</td>
<td>III C</td>
<td>0201-0501</td>
</tr>
<tr>
<td>Celiac 20</td>
<td>9</td>
<td>Female</td>
<td>24</td>
<td>nd</td>
<td>&gt;125</td>
<td>+</td>
<td>III B</td>
<td>0201-0301</td>
</tr>
</tbody>
</table>

AEMA, antiendomysial antibody; ATGA, anti-transglutaminase antibody, expressed as U mL⁻¹.
streptavidin–biotin universal detection system (Immunotech, Marseille, France). Sections were microwaved in 10 mM of citrate buffer (pH 6.0) for antigen retrieval and cooled in phosphate-buffered saline. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide. Sections were then treated with the protein-blocking agent, incubated with the primary antibody, followed by the biotinylated secondary antibody and the streptavidin–peroxidase reagent. The immune reaction was visualized with chromogen working solution AEC (Immunotech, France), and sections were counterstained with hematoxylin. Negative control experiments to ST2 and IL-33 immunostaining were performed by incubating sections with isotype-matched IgG1.

DNA extraction and HLA typing
DNA was extracted from peripheral blood using the QIAamp DNA mini-kit (Qiagen Iberia SL, Madrid, Spain). The identification of the main HLA haplotypes associated with CD was determined by CeliacStrip kit (Operon, Zaragoza, Spain) following the manufacturer’s instructions.

Peripheral blood mononuclear cell cultures
PBMCs from patients with active CD were isolated from 6 mL of heparinized blood by Histopaque gradient (Sigma Aldrich, Madrid, Spain) centrifugation and cultured at a density of 1 × 10^6 cells mL^-1 in RPMI-1640 culture medium (Gibco, Thermo Scientific, Madrid, Spain). After 48 h, the PBMCs were incubated with barley, gliadin (control positive), and rice prolamin peptides (50 μg mL^-1) for 24 h. Rice prolamin peptides was used as a negative control. Supernatants were collected and stored at −80°C.

Previously, we have found a direct correlation between the immunogenicity of the different barley varieties and the presence of the specific peptides with a higher/lower potential immunotoxicity (10–12). There is a wide range of variation in the potential immunotoxicity of barley cultivars that could be due to differences in the degree of immunogenicity in their sequences. These cultivars were chosen based on their previously reported high CD toxicity for our research group. The immunogenicity of different peptides was determined by T-cell proliferation and IFN-γ production in the cell culture.

Determination of serum and supernatants IL-33 and sST2 by ELISA
IL-33 and sST2 levels were assessed by ELISA assays in serum and PBMC supernatants from patients with CD and from healthy control using commercial detection systems (Abcam, Cambridge, UK) following the manufacturer’s instructions. For each assay, the standards as well as the samples were tested in duplicate. To estimate the cytokine concentration (pg mL^-1), we used a regression curve; the detection limit of human IL-33 was defined as 0.2 pg mL^-1 and less than 2.0 pg mL^-1 for ST2.

IFN-γ production
Supernatants from PBMC culture were collected after 24 h of peptide incubation and stored at −80°C for IFN-γ determination using a commercial ELISA kit in accordance with the manufacturer’s instructions (Thermo Scientific, Madrid, Spain). Standards were run on each plate. The sensitivity of the assay was <2 pg mL^-1.

Cell proliferation analysis
T-cell proliferation was determined after 48 h of incubation using the ELISA 5-bromo-2-deoxyuridine cell proliferation test (Millipore Chemicon, CA, USA). The stimulation index (SI) value was calculated by dividing the mean absorbance/10 at 450 nm after stimulation by the mean absorbance of T cells exposed to the culture medium alone (negative control) and divided by 10. The proliferation of PBMCs was expressed as the mean fluorescence intensity.

Statistical analysis
Each experiment was made in duplicate on separate days. Data are expressed as mean ± SD. All statistical analyses were performed with the STATGRAPHICS program. The differences between groups were examined by one-factor analysis of variance. When it was statistically significant, secondary Bonferroni-corrected t-tests were applied among groups. A statistical probability of p < 0.05 was considered significant.

RESULTS
The demographic, clinical, and immunological characteristics of CD patients are presented in Table 1.

Serological and histological analysis
Serological study. The AEMA was positive in all CD patients. The anti-transglutaminase antibody (ATGA) was elevated (range 160–800) in the 95% of CD patients. There were no patients with IgA deficiency.

Small-intestine biopsy. Patients with CD presented partial or total villus atrophy with increased numbers of intraepithelial infiltrated lymphocytes. Positive histology cases were classified considering the criteria of Marsh (types I–IV). Of the CD patients, 82% were found to have Marsh III lesions (partial or complete villous atrophy and crypt hypertrophy), 9% had Marsh IV (villi totally atrophied, i.e., completely flattened) hypoplasia, and 9% showed Marsh II lesions (crypt hyperplasia in addition to intraepithelial lymphocytosis; Table 1).

HLA-DQ2/DQ8 genotypes
HLA genotype frequencies among these patients with CD were as follows: HLA-DQ2 (DQA1*0501, DQB1*0201) was the most frequent allele in the patients (85%): DQ2 homo (5 patients; 29%), DQ2 hetero (12 patients; 71%). Three patients (15%) were carriers of DQ8 (DQA1*0301,*03:03–DQB1*03:02; Table 1).

ST2 and IL-33 distribution in intestinal mucosa tissue from CD patients
The diversity of cell types present in colon mucosa makes it necessary to develop a local detection of ST2 and IL-33 and to
evaluate the cellular source, which might provide an adequate interpretation of the results. For this, a immunohistochemistry technique was used.

IL-33 was expressed in epithelial cells in CD, and the expression pattern showed expression both in the surface epithelium as well as in crypt cells (Figure 1a). In addition to epithelial expression, IL-33 positive cells were also found in the lamina propria, in cells morphologically resembling lymphocytes. It was confirmed by immunohistochemistry that IL-33 is expressed in active CD, whereas more low staining levels were detected in specimens of Crohn’s disease. The expression pattern in the Crohn’s samples was restricted to epithelial cells and in lamina propria cells (Figure 1b). In control samples, we found low IL-33 epithelial expression (Figure 1e) in comparison with CD and Crohn’s disease samples.

Intense ST2 staining was detected in inflamed intestinal CD samples, although limited mainly to epithelial cells and Lieberkhn crypts. The expression pattern was observed in the cytoplasm and the apical surface of crypt epithelial cells (Figure 1c). We found low ST2 expression in lamina propria cells like lymphocytes. We observed a loss of ST2 staining in the epithelium and Lieberkhn crypts in the Crohn’s disease samples (Figure 1d). We found no ST2 expression in control patients, as no immunostaining in cells appeared at baseline level in the Lieberkhn crypts. No expression was found in epithelial cells and lamina propria (Figure 1f). An immune reactivity pattern was absent when isotype controls were used (data not shown).

Detection of ST2 and IL-33 protein in serum from CD patients

We evaluated the levels of IL-33 and ST2 in the serum of CD patients in order to identify potential biomarkers for the disease. IL-33 and ST2 levels significantly rose in the sera of

![Image](https://example.com/image1.png)

**Figure 1** Immunohistochemistry analysis: (a) Immunostaining for IL-33 in celiac disease patients (n = 20), showing expression in epithelial and lamina propria cells. (b) Immunostaining for IL-33 in Crohn’s disease patients (n = 7). (c) Immunostaining for ST2 in CD patients showing expression in Lieberkhn crypts. (d) Immunoreaction for ST2 in Crohn’s disease patients. (e) IL-33 expression in control patients (n = 8); (f) ST2 expression in control patients. Magnification: ×200.
CD patients in comparison with control and Crohn’s disease patients. As shown in Figure 2, the mean levels of IL-33 were 22.91 ± 8.73 pg mL⁻¹, 3.25 ± 1.75 pg mL⁻¹, and 6.92 ± 3.35 pg mL⁻¹ in sera, respectively, from CD patients, control and Crohn’s disease patients. The mean levels of sST2 were 81.33 ± 12.2 pg mL⁻¹, 21.98 ± 6.25 pg mL⁻¹, and 30.2 ± 7.20 pg mL⁻¹ in sera, respectively, from CD patients, control and Crohn’s disease patients.

**In vitro** PMBC cultures

We incubated PBMC from CD patients with the toxic fragments of gliadin peptides (barley and oat varieties previously purified to determine whether any of these epitopes stimulated IL-33/ST2 production). Under these conditions, PBMC produced IL-33 and ST2, demonstrating that the IL-33/ST2 response to peptides results from direct interactions between PBMCs and peptides. Our studies in vitro show that enzymatically digested wheat gliadin and toxic peptides of barley varieties significantly stimulates the production of IL-33 and sST2 in PBMC from CD patients as shown in Figure 3, implying a role for IL-33/ST2 axis in the pathogenesis of CD.

The release of IFN-γ into the culture medium after the exposure of celiac PBMCs to peptides was assessed (Figure 4). Higher levels of IFN-γ were found in the culture supernatants PMBCs of patients with active CD released IFN-γ in response to gluten stimulation. According to this assay, barley peptides were strongly immunogenic with the highest values of IFN-γ release.

We found a significant increase difference in cell proliferation in cultures incubated with toxic peptides of barley peptides. The sharp increase in PBMC proliferation was found in cultures incubated with barley peptides (SI = 17 ± 0.9), even more elevated than that produced by gliadin, although this increase was not significant. These results clearly indicate that toxic barley peptide displayed the highest activity and was the most potentially immunogenic (Figure 4). We found a significant decrease in cell proliferation in the cultures without toxic peptide incubation (control).

**DISCUSSION**

Increasing evidence has shown that IL-33 and its receptor ST2 contribute to the pathogenesis of various autoimmune diseases. However, no information is available on the role of IL-33 and sST2 in CD in comparison to other autoimmune diseases. This is the first report available to demonstrate the presence of higher levels of IL-33 and ST2 in serum and mucosal samples of CD patients than in patients with Crohn’s disease and control subjects. The results obtained by other authors regarding IL-33/ST2 expression patterns in the gut mucosa as

![Figure 2](image1.png)  
**Figure 2** IL-33 and sST2 expression in the serum of celiac disease, control health, and Crohn’s disease patients. IL-33 and sST2 were highly expressed in the serum from celiac disease patients (n = 20) in comparison with control (n = 8) and Crohn’s disease (n = 7). Significantly different at *p < 0.05 are shown.

![Figure 3](image2.png)  
**Figure 3** IL-33 (a) and sST2 levels (b) in culture supernatants of PBMCs (n = 15) with different toxic peptides in comparison with control (rice). Significantly different at **p < 0.005 are shown.
well as the systemic circulation of IBD patients were also confirmed by our study in CD. IBD and CD, both are complex diseases with genetics and environment contributing to dysregulation of innate and adaptive immune responses, leading to chronic inflammation and disease. We, also, show for the first time that toxic peptic fragments of wheat gliadin and barley peptides stimulate the production of IL-33/ST2 in in vitro cultures of PBMCs.

IL-33 demonstrates a potent pro-inflammatory effect, exacerbating the disease states, particularly through the recruitment of activated immune cells to the inflammation site and the production of several pro-inflammatory cytokines. From lamina propria immune cells, enhancing both Th1 and Th2 responses, being a regulator of inflammation and helper T1 (TH1)/TH2 balance. IL-33 may present a general amplifier of inflammation dependent of local cellular and extracellular context. As such, IL-33 may represent a primary, innate cytokine capable of inducing pathogenic, adaptive immune responses in the gut.

Damaged, stressed, or necrotic cells in CD can potentially release IL-33 as a danger signal/alarm in to alert the immune system of a local threat. In fact, several genetic studies have indicated the loss of epithelial barrier integrity as a possible trigger for the development of pathologic inflammatory gut responses in CD patients. IL-33 has the ability to signal local innate immune responses in an effort to mount an effective, physiological inflammatory reaction to restore normal gut homeostasis. Also, IL-33 may have a pro-inflammatory effect on lamina propria immune cells while at the same time promoting wound healing and epithelial repair when acting on epithelial cells. In our study, lamina propria cells are IL-33 sources in CD.

In vivo studies have demonstrated that LPS-stimulated murine macrophages raise IL-33 transcript and protein levels in the extracellular medium. In this sense, mast cells and basophils are the main IL-33 targets in lamina propria, as surface ST2 expression is increased by IL-33 stimulation, and both cells secrete Th2 cytokines and chemokines when stimulated by IL-33. We have shown the release of IL-33 protein after stimulation of different cell types by a variety of stimuli, such as toxic gliadin peptides in CD patients, showing that IL-33 can be released like other members of the IL-1 family acting as a classical cytokine and a stress or danger signal. It can be hypothesized that, because of either intrinsic defects (e.g., structural impairment affecting cell integrity, barrier dysfunction) or extrinsic exposure to harmful stimuli, as in the case of gluten exposure, stressed gut epithelial cells can release potent levels of IL-33 as a danger signal, initiating an inflammatory cascade that leads to intestinal inflammation.

The data presented point to a possible important inter-regulation of IFN-γ and IL-33 in CD. IFN-γ can induce IL-33 expression and may contribute to its release by its pop-apoptotic effect in the intestinal mucosa. When IL-33 is released from cells, it could act on T cells to produce more IFN-γ. We thus propose considering IL-33 not only as a dual-function cytokine with regard to its cytoplasmic and nuclear function but also with regard to its action on either Th1- or Th2-linked inflammation. IL-33 synergizes with TCR signaling and IL-12 in promoting IFN-γ production and the effecter function of CD8+ T cells. ST2 is also found in NK and NKT cells, which respond to IL-33 with increased IFN-γ production, suggesting a role for IL-33/ST2 in innate Th1 type immune responses. Bourgeois et al. found that IL-33 is a potent co-stimulator of iNKT cells that induces, in combination with IL-12, a preferential increase of IFN-γ production in vivo. Our preliminary data appear to indicate that IL-33 can also amplify IFN-γ production by conventional CD4+ memory T cells, and we show a new pro-Th1 activity of IL-33, which can act as a co-stimulatory factor in innate cellular immune responses. Our findings challenge the prevailing opinion that IL-33 is strictly a pro-Th2 cytokine and provide further evidence for the importance of the microenvironment and the cellular context for determining both Th1- and Th2-oriented immune responses.

The data presented by Lecart et al. demonstrate that the T1/ST2 gene product is inducible protein, and in culture supernatants of in vitro activated Th2 clones contained variable but...
significant levels of ST2 protein. However, the finding that peak levels of ST2 production by Th2 clones in vitro were lower than those measured in serum samples from asthmatic patients in crisis suggested to these authors that other cell types, most likely eosinophils, contribute to ST2 production in vivo as well.

In addition, it has not been addressed whether soluble ST2 is associated with IL-33 signaling. In this sense, soluble ST2 may have a negative function in IL-33 signaling. Several studies have shown that soluble forms of cytokine receptors function as positive or negative regulators in the expression of cytokines and growth factors. Serum levels of soluble ST2 have been found to be high in various diseases such as rheumatoid arthritis, systemic lupus erythematosus, idiopathic pulmonary fibrosis, and inflammatory bowel disease, as well as asthma. Soluble ST2 may participate in the regulation of inflammatory cytokines besides Th2 cytokines.

**CONCLUSION**

The findings of our study show elevated serum and tissue levels of IL-33 and sST2 in patients with CD, suggesting that the IL-33/ST2 system has a role in the pathogenesis of this disease and may represent potential biomarkers in monitoring autoimmune disease activity and subsequent efficacy of clinical treatment. Further studies will be necessary in order to thoroughly investigate the exact role of IL-33/ST2 axis in human CD.

**FUNDING STATEMENT**

This work was supported by AGR2009-4966M (Proyecto de Excelencia, Junta de Andalucía, and Research group BIO220, Junta de Andalucía).

**ACKNOWLEDGEMENTS**

The authors are grateful to Dr. Sousa and Dr. Cebolla for the barley, gliadin, and rice prolamins peptides of the in vitro assays.

15. **Liew FY, Pitman NI, Mclinnes IB.** Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* 2010; 10: 103–110.
17. **Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L et al.** IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc Natl Acad Sci USA* 2007; 104: 282–287.
30 Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005; 23: 479–490.


