Clin Exp Immunol 1996; **103**:295–303

Gluten activation of peripheral blood T cells induces a Th0-like cytokine pattern in both coeliac patients and controls

E. M. NILSEN, H. A. GJERTSEN*, K. JENSEN,* P. BRANDTZAEG & K. E. A. LUNDIN*† *Laboratory for Immunohistochemistry and Immunopathology (LIIPAT), Institute of Pathology, *Institute of Transplantation Immunology, and †Medical Department A, University of Oslo, The National Hospital, Rikshospitalet, Oslo, Norway*

(Accepted for publication 29 September 1995)

SUMMARY

Coeliac disease is apparently a T cell-mediated disease, precipitated in the proximal small intestine of susceptible individuals by gluten. Preferential presentation of gluten peptides most probably takes place in coeliac mucosa by the disease-associated HLA-DQ2 and -DQ8 molecules. In peripheral blood, however, both HLA-DR, -DQ and -DP-restricted T cell responses to gluten have been observed. We examined gluten-specific T cell clones (TCC) derived from peripheral blood for cytokine production to see if their profiles were related to the HLA restriction or the disease state of the donors. As previously found for mucosal TCC, the main product was interferon-gamma (IFN-γ), often with additional IL-4, IL-5, IL-6, IL-10, tumour necrosis factor, and transforming growth factor-beta. Regardless of restriction element or disease state, gluten-reactive TCC from peripheral blood therefore seem to secrete cytokines compatible with a Th0 profile.

Keywords peripheral blood T cell clones coeliac disease T cell subset cytokines

INTRODUCTION

Coeliac disease is a malabsorption disorder characterized by crypt cell hyperplasia and villus atrophy [1,2]. It is probably an immune-mediated disease, precipitated in susceptible individuals by ingestion of wheat gluten and related prolamines from other cereals. Coeliac disease shows a strong HLA association, predominantly with a particular DQ heterodimer, DQ2 (DQα1*0501, β1*0201), and apparently DQ8 (DQα1*0302, β1*0301) in a small subset [3,4]. Gluten-specific mucosal CD4$^+$ T cells employing the α/β T cell receptor seem to be central in the immunopathology of this disease [5–8]. Importantly, such cells show a strikingly predominant restriction for the disease-associated DQ2 and DQ8 molecules [6,7].

Previous studies have reported proliferative responses of circulating T cells to gluten peptides, both in healthy controls and coeliac patients [9–11]. Gluten-reactive peripheral blood T cell clones (TCC) were recently established from such patients [12,13] as well as from healthy individuals [14], after stimulation with gluten peptides *in vitro*. Such TCC were obtained from four coeliac patients and from four controls, with a range of HLA-DR, -DQ and -DP molecules as defined restriction elements. Because secretion of cytokines may be involved in the pathogenesis of coeliac disease, we wanted to characterize further the cytokine profiles of gluten-reactive TCC. Our findings demonstrated that such TCC obtained from peripheral blood secrete cytokines with a Th0-like profile and, as for their mucosal counterparts, interferon-gamma (IFN-γ) is the major product.

Correspondence: Ellen M. Nilsen, LIIPAT, Rikshospitalet, N-0027 Oslo, Norway.

PATIENTS AND METHODS

Patients and healthy controls

The patients used as T cell donors were all diagnosed according to the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) criteria and had been on a gluten-free diet for several years [12]. The controls were healthy blood donors, or members of the hospital staff. They had no clinical signs of coeliac disease and were negative for antibodies against gliadin as well as endomysium [14]. Small intestinal biopsies from control individual no. 5 were completely normal [14].

T cell clones

Gluten-reactive peripheral blood TCC were prepared from four treated coeliac patients and four healthy individuals, as detailed elsewhere [12–14]. All carried HLA-DR3 (DRB1*0301) as well as HLA-DQ2 (DQA1*0501, DQB1*0201) and in most cases a non-DR3, DQ2 haplotype. The cytokine profiles of 21 TCC from four coeliac patients and six TCC from two healthy controls were analysed. Eight TCC reactive with *Mycobacterium tuberculosis* were included as controls; these clones were established from the peripheral blood of coeliac patient no. 1 (Lundin *et al*. unpublished).

© 1996 Blackwell Science

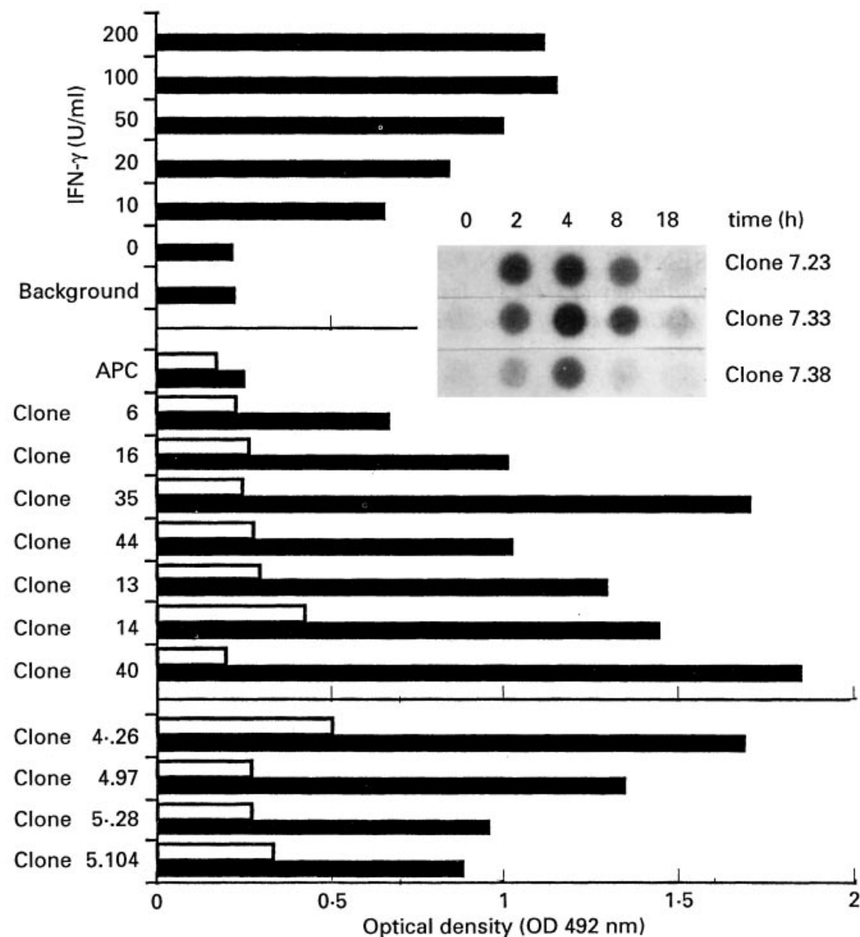

Fig. 1. Cell ELISA (CELISA) measurements of HLA-DR expression by HT-29.E10 cells after stimulation for 48 h with supernatants (diluted 1:20) from various gluten-stimulated (middle panel) or *Mycobacterium tuberculosis*-stimulated (lower panel) T cell clones (■) as indicated in relation to antigen-presenting cells (APC) alone, clonal control without relevant antigen (□), and reference values obtained with 0–200 U/ml of rhIFN-γ (■) (upper panel). Results presented as mean OD at 492 nm of triplicates tested in the same experiment (repeated in a reproducible manner at least twice). Insert shows autoradiogram of slot-blot hybridization results with antisense DNA probe for IFN-γ mRNA from three gluten-stimulated (2–18 h) T cell clones as indicated, compared with unstimulated control (0 h).

Preparation of TCC supernatants and proliferative assays
The TCC (5×10^5 cells) were stimulated with a peptic-tryptic digest of gluten (2 g/*l*) in the presence of antigen-presenting cells (APC) that expressed the relevant HLA class II restriction element. The latter were Epstein–Barr virus (EBV)-transformed B cells (1×10^6, irradiated at 100 Gy), and incubation took place in 1·5–2 ml medium (RPMI 1640 containing 15% inactivated human serum and antibiotics). Supernatants from parallel cultures without gluten served as negative controls. In preliminary experiments supernatants were collected after 12, 24, 36 and 48 h of stimulation; the latter was chosen as the most optimal time point (except for IL-2 which was found after 6 h). The samples were cleared by centrifugation and stored as aliquots at $-70°C$.

Mycobacterium tuberculosis-reactive TCC were prepared similarly, with purified protein derivative (PPD) of *Myco. tuberculosis* (4 mg/*l*) as antigen, in the presence of peripheral blood mononuclear cells (PBMC) as APC.

Proliferative assays of TCC were tested with 2×10^4 T cells stimulated in triplicates with 5×10^4 irradiated APC in the presence or absence of 2 g/*l* of gluten digest or 4 mg/*l* of *Myco. tuberculosis* [12].

Standards, probes, and primer sets for cytokines
The cytokines were recombinant (r) human (h) IFN-γ, rh tumour necrosis factor-alpha (TNF-α), rh transforming growth factor-beta (TGF-β), rhIL-5 and rhIL-6 obtained from Genzyme Corporation (Cambridge, MA), and rhIL-2 from Amersham International (Aylesbury, UK). Activity units (U) were based on information given by the manufacturers. The single-stranded antisense DNA probes, as well as the primer set sequences used in polymerase chain reaction (PCR), were previously described [15]. All primer sequences were derived from separate exons of the gene sequences, thus spanning intron(s); amplification products of genomic DNA could therefore be distinguished from products of reverse transcribed mRNA.

Bioassays for cytokines
IL-2, IL-5 and IL-6 activities were measured in proliferative bioassays with the murine cell lines HT-2, LYH7.B13 or B9, respectively. These assays and their specificities have been detailed elsewhere [15–18]. TNF (α and β) and TGF-β activities were measured in cytotoxic bioassays with the murine cell line WEHI 164 clone 13 and in the mink lung cell line CCL-64, respectively [15,19,20].

Cell ELISA for epithelial secretory component and HLA-DR
Total cellular secretory component (SC) (or polymeric immunoglobulin receptor (pIgR)) and HLA-DR expression were determined by a semiquantitative cell ELISA (CELISA) as detailed elsewhere [15,21]. CELISA for HLA-DR expression was primarily used as a bioassay to measure IFN-γ. Induction of HLA-DR in HT-29 cells is known to be a selective function of IFN-γ [22], although subsequent up-regulation of HLA-DR can be synergistically enhanced by TNF-α [23].

Immunoassays for cytokines
Commercial ELISA kits were used according to the recommendations of the manufacturers for immunological quantification of hIL-2 (Amersham) and hIL-4 (Amersham; Genzyme Corporation) as well as hIL-10 (Medgenix Diagnostics, Brussel, Belgium).

TCC stimulation for mRNA analysis
Total RNA from three TCC (clones 7.23, 7.33 and 7.38) was isolated by the guanidinium isothiocyanate method [24] and quantified by spectrophotometry. RNA was extracted at each time point from 5×10^6 T cells after incubation with 5×10^6 APC in the presence of gluten digest for 0, 2, 4, 8 and 18 h.

PolyA$^+$ RNA from four TCC (clones 2.19, 2.25, 1.17 and 1.22) was isolated with Dynabeads Oligo(dT)$_{25}$(M-280; Dynal, Oslo, Norway). PolyA$^+$ RNA was extracted from $0{\cdot}5 \times 10^6$ T cells after incubation with $1{\cdot}5 \times 10^6$ APC in the presence of gluten digest for 0, 4 and 8 h.

Cytokine mRNA analysis by slot-blotting
Nylon filters (Schleicher & Schuell, Dassel, Germany) were inserted in a slot-blot apparatus (SRC 96 Minifold II; Schleicher & Schuell). Samples of total RNA (6 μg) were applied, and the filters were next processed as described earlier [15].

PCR for cytokine mRNA
PolyA$^+$ RNA was extracted (see above) for semiquantitative analysis of cytokine mRNA from four TCC (clones 2.19, 2.25, 1.17 and 1.22). The RNA was subjected to reverse transcription (RT), and amplified by cytokine-specific primers as described earlier [15]. The numbers of cycles used for amplification of each cytokine are described in the legend to Fig. 2. The size of the PCR products, the primer set sequences, and the annealing temperatures for each primer have been reported elsewhere [15].

RESULTS

Expression and secretion of IFN-γ
CELISA for HLA-DR expression in HT-29 cells was primarily used as a bioassay to measure IFN-γ in the TCC supernatants [22]. Figure 1 shows the results of a representative experiment with supernatants from seven gluten-reactive and four *Myco. tuberculosis*-reactive TCC after stimulation in the presence of APC. The overall CELISA results (Table 1) showed that all of the 21 gluten-reactive TCC from coeliac patients secreted considerable amounts of IFN-γ after gluten stimulation, most of the levels being remarkably high (>1000 U/ml). However, in clone 7.23 induction of IFN-γ was seen only after addition of exogenous IL-2 to the culture. The six gluten-reactive TCC from healthy individuals also produced much IFN-γ, and the same was true for TCC reactive with *Myco. tuberculosis* (Table 1). This was likewise evidenced by expression of SC in HT-29 cells, by all clones after gluten stimulation (Table 1). Induction of SC in this cell line is mainly (but not solely) an effect of IFN-γ [23].

Slot-blot analysis of RNA extracted from three gluten-reactive TCC from coeliac patients revealed very little IFN-γ mRNA in unstimulated cells (time 0), but showed striking up-regulation of this message after exposure to the gluten digest in the presence of APC for 2–4 h, thereafter decreasing (Fig. 1). Semiquantitative PCR performed on four TCC isolated from healthy individuals demonstrated clearly IFN-γ mRNA in three of them after gluten stimulation for 4–8 h, but only hardly detectable levels in the fourth (Fig. 2).

Expression and secretion of TNF
Most stimulated TCC (18 of 21) from coeliac patients secreted TNF in the range of 10–400 U/ml (Table 1) measured by a bioassay responding to both TNF-α and TNF-β. The five tested TCC from healthy individuals secreted TNF in the range of 9–125 U/ml (Table 1). All of the *Myco. tuberculosis*-reactive TCC were either negative or secreted only small amounts (Table 1). Figure 3 shows data obtained by bioassay for supernatants from nine gluten-reactive and four *Myco. tuberculosis*-reactive TCC after antigen stimulation in the presence of APC. Slot-blot analysis of three TCC from coeliac patients demonstrated TNF-α mRNA in two after 2–8 h of stimulation (Fig. 3). Semiquantitative PCR demonstrated TNF-α mRNA in all of the four tested TCC from healthy individuals after 4–8 h of gluten stimulation, and low levels in unstimulated cells as well (Fig. 2).

Expression and secretion of TGF-β
Supernatants from 10 gluten-reactive TCC (five from coeliac patients and five from healthy individuals) contained TGF-β in the range of 40–1000 pg/ml as measured by bioassay, both with and without gluten stimulation (Table 1). Semiquantitative PCR on four TCC from healthy individuals likewise demonstrated TGF-β mRNA in stimulated as well as unstimulated cells (Fig. 2).

Expression and secretion of IL-2
Neither bioassay nor ELISA revealed IL-2 in undiluted supernatants of stimulated TCC (both gluten- and *Myco. tuberculosis*-reactive) after 48 h, whereas up to 2 U/ml was detected in most of the gluten-stimulated TCC after 6 h (data not shown). Semiquantitative PCR performed on four TCC from healthy individuals demonstrated IL-2 mRNA in all after gluten stimulation for 4–8 h, while unstimulated cells were negative or only weakly positive (Fig. 2).

Expression and secretion of IL-4
After gluten stimulation, all of the 12 tested TCC from coeliac patients secreted IL-4 as detected by ELISA, although the levels varied considerably (Fig. 4, Table 1). Slot-blot analysis of two such TCC showed no IL-4 mRNA in unstimulated cells (time 0) but striking up-regulation after exposure to gluten digest in the presence of APC for 2–8 h, decreasing after 18 h (Fig. 4). Much less message was detected in a third (clone 7.38), in agreement with the ELISA results (Table 1). Semiquantitative PCR demonstrated IL-4 mRNA in all of the four tested TCC from healthy individuals after gluten stimulation for 4 h (Fig. 2), in agreement with the ELISA results (Table 1). Only one TCC (clone 1.19) was negative for IL-4 by ELISA (Table 1). Conversely, all of the four

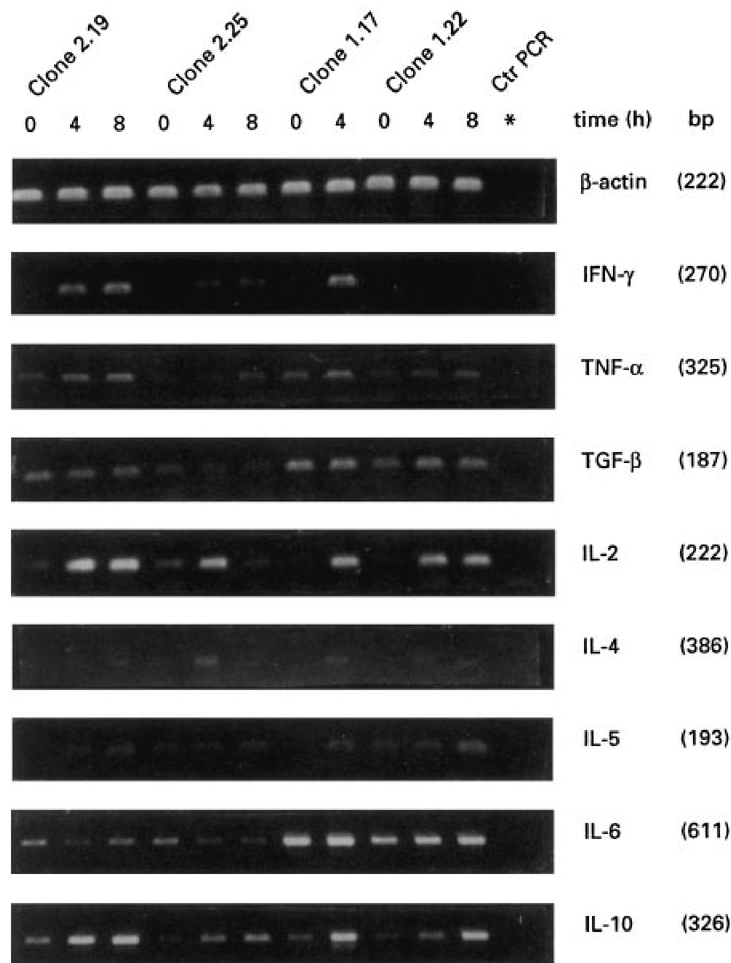

Fig. 2. Agarose electrophoresis for semiquantitative determination of polymerase chain reaction (PCR)-amplified cytokine mRNA in four T cell clones 4 and 8 h after gluten stimulation, in relation to unstimulated controls (0). PolyA$^+$ RNA was reverse-transcribed to cDNA. Amplification was performed by cytokine-specific primers with 1 μl cDNA (β-actin or IFN-γ) for 25 cycles, 1 μl cDNA (tumour necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β) or IL-10) for 35 cycles, 3 μl cDNA (IL-4, IL-5 or IL-6) for 35 cycles, or 3 μl cDNA (IL-2) for 40 cycles. The indicated size (bp) of the amplification product matched what was predicted from the position of the primer pairs.

tested *Myco. tuberculosis*-reactive TCC were negative for this cytokine by ELISA (Table 1).

Expression and secretion of IL-5

After gluten stimulation, 11 of the 20 TCC from coeliac patients tested by bioassay secreted IL-5 in the range 30–400 ng/ml (Table 1, Fig. 5). Slot-blot analysis revealed little IL-5 mRNA in unstimulated cells (time 0) and after 2 h of stimulation, but showed striking up-regulation in clone 7.23 after exposure to gluten-peptides in the presence of APC for 4–8 h, decreasing after 18 h (Fig. 5). Considerably less IL-5 mRNA was detected for clones 7.33 and 7.38. Four of the six TCC from healthy individuals secreted IL-5 in the range 20–200 ng/ml. Semiquantitative PCR demonstrated low levels of IL-5 mRNA in these TCC after gluten stimulation for 4–8 h, and also in two unstimulated clones (Fig. 2). All the *Myco. tuberculosis*-reactive TCC were negative for IL-5 by bioassay (Fig. 5, Table 1).

Expression and secretion of IL-6

After gluten stimulation, 16 of 21 TCC from coeliac patients tested by bioassay secreted IL-6 in the range 25–500 U/ml (Table 1). By contrast, the *Myco. tuberculosis*-reactive TCC secreted only low levels of IL-6, as was also the case for the TCC from healthy individuals (Table 1). Semiquantitative PCR performed on four TCC from healthy individuals demonstrated IL-6 mRNA in stimulated as well as in unstimulated cells (Fig. 2).

Expression and secretion of IL-10

After gluten stimulation, seven of the nine tested TCC from coeliac patients secreted small amounts of IL-10 as detected in undiluted supernatants by ELISA, whereas one secreted >1500 pg/ml and one was negative (Table 1). Three of the TCC from healthy individuals secreted substantial amounts of IL-10, whereas one was negative. Semiquantitative PCR on four of these TCC demonstrated IL-10 mRNA in all of them after gluten stimulation for 4–8 h, whereas two unstimulated TCC showed low expression and two were negative. The only *Myco. tuberculosis*-negative TCC tested for IL-10 was negative (Table 1).

DISCUSSION

This study reports for the first time cytokine profiles of cloned

Table 1. Cytokine secretion profiles of various peripheral blood gluten-specific T cell clones (TCC) compared with TCC reactive with *Mycobacterium tuberculosis*

					CELISA		Bioassay				Immunoassay	
Subject* no.	T cell clone	Stimulation	Proliferation (ct/min)	HLA restriction	HLA-DR (U/ml IFN-γ)	SC (U/ml IFN-γ)	IL-5 (ng/ml)	IL-6 (U/ml)	TGF-β (pg/ml)	TNF (U/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
1	7·23	Gluten	15 280	DQ2	Negative	500	Negative	25	ND	120	2000	ND
(CD)	7·23	Gluten + IL-2	ND""	DQ2	100	>1000	ND	65	ND	30	ND	ND
	7·38	Gluten	46 968	DQ2	10 000	>2000	ND	40	ND	400	180	ND
	7·38	Gluten + IL-2	ND	DQ2	>10 000	>2000	ND	100	ND	600	ND	ND
	8·19	Gluten	ND	DQ2	2000	1500	50	>500	ND	250	ND	ND
	8·26	Gluten	ND	DQ2	1000	1000	>400	>500	1000	260	1000	20
	33 non-Tα	Gluten	20 593	DQ2	2000	2000	30	500	ND	10	1000	ND
	8·14	Gluten	9949	DR3	500	1000	30	Negative	ND	Negative	ND	ND
	8·23	Gluten	45 128	DR3	2000	>2000	100	50	600	Negative	1000	100
2	16	Gluten	10 640	DQ2	1000	2000	50	400	1000	80	250	140
(CD)	28	Gluten	6454	DQ2	200	1200	Negative	>500	ND	20	ND	20
	44	Gluten	41 321	DQ2	1000	1500	Negative	400	ND	200	35	ND
	6	Gluten	10 574	DR3	200	1500	Negative	Negative	ND	10	ND	120
	35	Gluten	5300	DR3	>2000	1500	60	>500	800	130	60	80
3	13	Gluten	ND	DQ2	>2000	2000	30	400	1000	40	125	Negative
(CD)	22	Gluten	4588	DQ2	200	500	Negative	Negative	ND	15	ND	ND
	40	Gluten	48 747	DQ2	>2000	1500	40	>500	ND	40	60	ND
	39	Gluten	5960	DR3	200	1500	Negative	50	ND	15	ND	80
	14	Gluten	4334	DR7	>2000	1500	Negative	>500	ND	30	30	ND
4	17	Gluten	9048	DR3	100	500	Negative	Negative	ND	Negative	ND	ND
(CD)	41	Gluten	75 667	DR3	>2000	>2000	125	Negative	ND	250	ND	ND
	21	Gluten	108 405	DR4	1000	>1000	Negative	500	ND	40	ND	>1500
	23	Gluten	123 364	DR4	1000	>1000	30	500	ND	80	250	ND
5	1·8	Gluten	ND	DQ2	40	600	40	5	800	100	ND	400
(Con.)	2·19	Gluten	4256	DQ2	800	400	>200	3	800	125	300	>1500
	2·25	Gluten	17 839	DQ2	700	>1500	120	2	300	60	500	>1500
6	1·19	Gluten	ND	DQ2	400	1200	20	2	ND	ND	Negative	Negative
(Con.)	1·17	Gluten	15 591	DR4	500	500	Negative	ND	60	9	300	ND
	1·22	Gluten	1397	DR4	400	400	Negative	ND	40	10	250	ND
1	4·26	Myco. tuberculosis	39 333	DR3	>2000	2000	Negative	50	ND	10	Negative	ND
(CD)	4·46	Myco. tuberculosis	22 360	DR3	>2000	1500	Negative	Negative	ND	30	ND	ND
	4·97	Myco. tuberculosis	28 844	DR3	>2000	2000	Negative	30	ND	10	Negative	Negative
	5·104	Myco. tuberculosis	13 783	DR3	500	1500	Negative	30	ND	10	ND	ND
	5·28	Myco. tuberculosis	14 782	DR3	1000	1500	Negative	50	ND	Negative	Negative	ND
	5·52	Myco. tuberculosis	13 737	DR3	1000	1500	Negative	60	ND	Negative	ND	ND
	5·65	Myco. tuberculosis	13 478	DR3	300	1500	Negative	100	ND	Negative	ND	ND
	5·95	Myco. tuberculosis	20 555	DR3	500	2000	Negative	50	ND	50	Negative	ND

Twenty-one TCC from four coeliac disease (CD) patients, and six TCC from two healthy controls (Con.) were investigated. Cell culture supernatants were prepated and tested after 48 h stimulation with gluten peptides in the presence of Epstein–Barr virus (EBV)-transformed B cells used as antigen-presenting cells (APC). Eight TCC reactive with *Myco. tuberculisis* established from peripheral blood of a coeliac disease patient (no. 1) were also included.

ND, Not determined.

© 1996 Blackwell Science Ltd, *Clinical and Experimental Immunology*, **103**:295–303

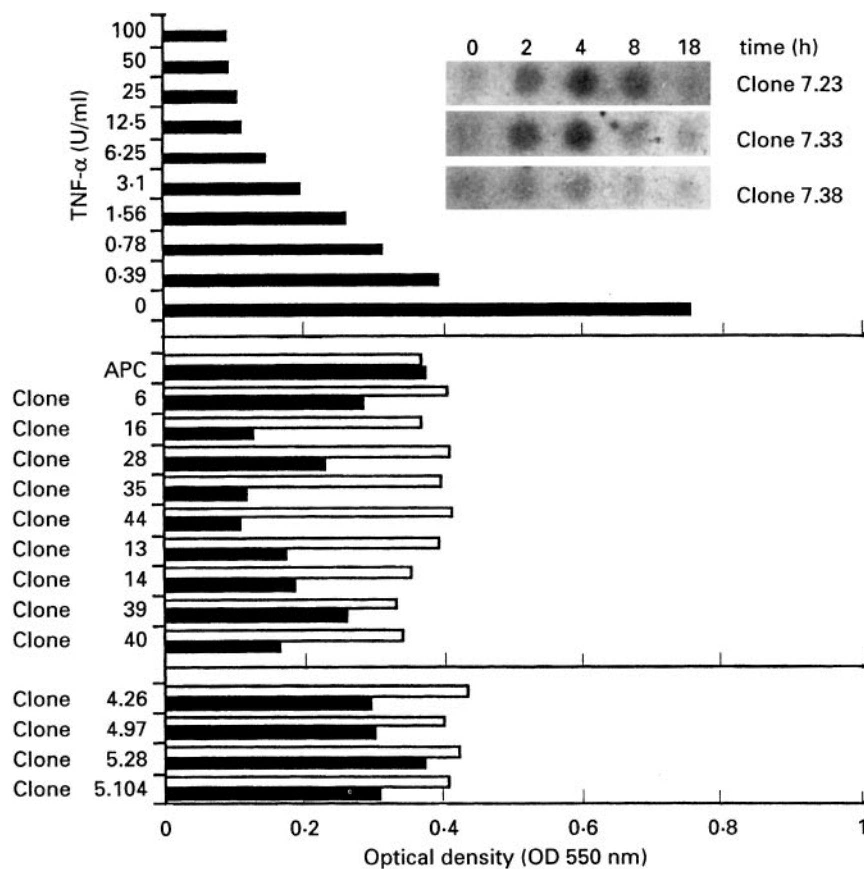

Fig. 3. Cytotoxic bioassay of tumour necrosis factor (TNF) activity in supernatants (diluted 1:10) from various gluten-stimulated (middel panel) or *Myco bacterium tuberculosis*-stimulated (lower panel) (48 h) T cell clones (■) as indicated in relation to antigen-presenting cells (APC) alone, clonal control without relevant antigen (□), and reference values obtained with 0–100 U/ml of rhTNF-α (■) (upper panel). Results presented as mean reduction of OD at 550 nm of triplicates tested in the same experiment (repeated in a reproducible manner at least twice). Insert shows autoradiogram of slot-blot hybridization results with antisense DNA probe for TNF-α mRNA from three gluten-stimulated (2–18 h) T cell clones compared with unstimulated control (0 h).

gluten-reactive $CD4^+$ T cells from peripheral human blood. After an *in vitro* gluten challenge, cells were isolated from four treated (gluten-free diet) patients with coeliac disease and from four healthy controls [12–14]. Of the 21 gluten-reactive TCC established from the coeliac patients, 11 were restricted by the disease-associated HLA-DQ2 heterodimer, whereas 10 were HLA-DR-restricted (both DR3, DR4 and DR7); of the six studied gluten-reactive TCC established from two healthy individuals, four were DQ2-restricted, whereas two were DR4-restricted.

Circulating T cells responsive to gluten have previously been detected in coeliac patients on a gluten-free diet as well as in healthy individuals, the strongest reactivity being observed in the patients [9–12]. We recently reported that gluten-specific TCC from peripheral blood of coeliac patients may be restricted by both DR, DQ and DP molecules [12], in contrast to our mucosal TCC that were restricted only by HLA-DQ2 (or HLA-DQ8 in a small subgroup) [6,7]. The mucosal TCC isoplated from coeliac mucosa produced cytokines with a Th1 or Th0 profile, IFN-γ being the predominant product [15]. In addition, most of the mucosal TCC produced variable levels of TNF and IL-6, and some secreted TGF-β, IL-4, IL-5 and IL-10 as well.

In the present study, the cytokine profiles of peripheral blood TCC were related to the actual HLA restriction element and the clinical state of the donor. Regardless of restriction element, all gluten-reactive peripheral blood TCC isolated from coeliac patients produced large amounts of IFN-γ after antigen stimulation, as previously shown for the DQ-restricted mucosal TCC [15]. Most of the peripheral blood TCC secreted variable levels of TNF, TGF-β, IL-4, IL-5, IL-6, and IL-10 as well. This pattern was compatible with a Th0 profile. Except for TGF-β and IL-6, no cytokine secretion was detected in the absence of gluten stimulation. The EBV-transformed B cells used as APC were likewise negative. The six TCC from healthy controls also secreted large amounts of IFN-γ (up to 800 U/ml), although consistently less than most counterparts from the coeliac patients (Table 1). They moreover produced variable amounts of the other cytokines, which was supported by examination of mRNA expression.

Previous studies have shown that HLA restriction may influence significantly the cytokine profiles of T cells [25,26]. Our mucosal DQ2-restricted TCC secreted cytokines mostly compatible with a Th1 pattern, whereas the smaller subset of DQ8-restricted TCC appeared to fit better with a Th0 profile [15]. However, because our DQ2-restricted TCC were derived from only two coeliac patients and the DQ8-restricted TCC from a single one, no firm conclusions can be drawn. Our present data on peripheral blood TCC restricted by DQ2, DR3, DR4 or DR7 did

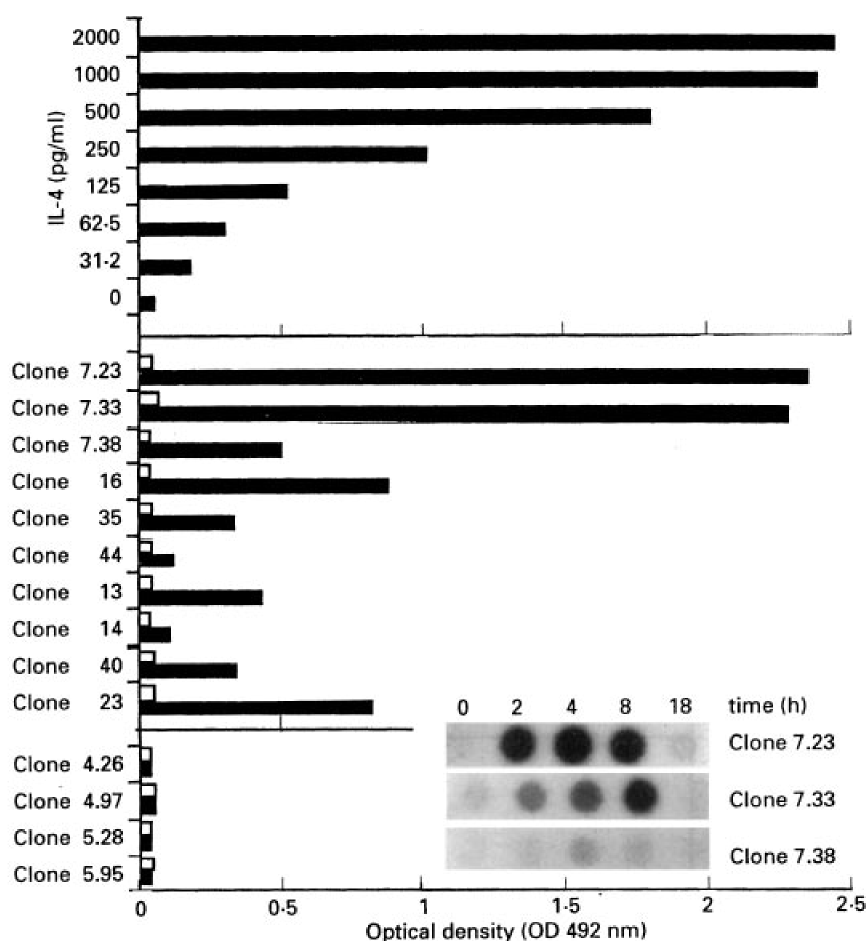

Fig. 4. ELISA measurements of IL-4 in supernatants (undiluted) from various gluten-stimulated (middel panel) or *Mycobacterium tuberculosis*-stimulated (lower panel) (48 h) T cell clones (■) as indicated in relation to clonal control without relevant antigen (□), and reference values obtained with 0–2000 pg/ml of rhIL-4 (■) (upper panel). Results are presented as mean OD at 492 nm of duplicates tested in the same experiment. Insert shows autoradiogram after slot-blot hybridization with antisense DNA probe for IL-4 mRNA from three gluten-stimulated (2–18 h) T cell clones as indicated, compared with unstimulated control (0 h).

not suggest that the HLA restriction element significantly dictates the cytokine profile.

The discovery of two major subsets of $CD4^+$ T cells (Th1 and Th2) and their involvement in different diseases [27,28] is of great clinical interest. Production of classical Th1 cytokines (IFN-γ, TNF and IL-2) and Th2 cytokines (IL-4, IL-5 and IL-10) has recently been studied in infectious [29,30] and allergic [31,32] disorders. Specific immune induction of cytokine secretion should yield the most accurate information regarding the antigen-specific Th cell population in a given disease. Evidence is emerging that the type of stimulatory antigen plays a major role in determining the cytokine profile of reactive T cells. Thus, human TCC obtained from peripheral blood of an atopic patient showed a Th1-like profile in response to mycobacteria, but a Th2-like profile in response to allergen [28]. Most of the gluten-reactive TCC analysed in our study produced cytokines with a Th0 profile, regardless of whether they were derived from coeliac patients or from healthy individuals. Conversely, the *Myco. tuberculosis*-reactive TCC obtained from one of the coeliac patients showed a clear Th1 profile with no detectable IL-4 and IL-5.

Taken together, our findings suggest that gluten peptides preferentially induce a Th0-like cytokine pattern in peripheral blood, both in coeliac patients and healthy individuals, regardless of the actual HLA (DR or DQ) restriction of the responding T cells. Why DQ restriction and to some extent a Th1-like cytokine profile are favoured in the coeliac lesion remains to be established.

ACKNOWLEDGMENTS

This study was supported by the Norwegian Cancer Society, the Research Council of Norway, and Pronova. E. M. N. is a Research Fellow of the University of Oslo. We thank Drude Hansson, Guri Jørum, Kari G. Lislerud, Tone Narvessen and Bjørg Simonsen for invaluable technical assistance. T. Espevik, T. Lea, K. E. Mostov and R. Palacios are acknowledged for the generous gifts of cell lines, and I. Moro for providing some of the primer sets for PCR. We thank Ludvig M. Sollid, Helge Scott and Erik Thorsby for valuable advice.

REFERENCES

1 Trier JS. Celiac sprue. New Engl J Med 1991; **325**:1709–19.
2 Marsh MN. Gluten, major histocompatibility complex, and the small intestine. Gastroenterology 1992; **102**:330–54.

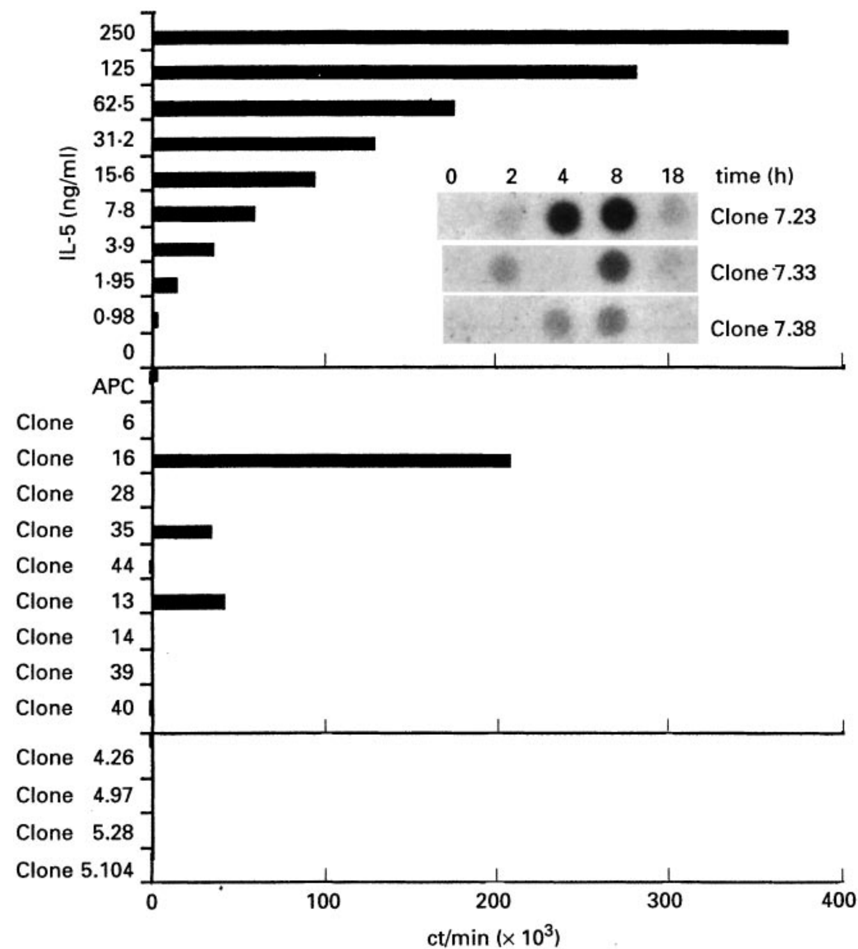

Fig. 5. Proliferative bioassay of IL-5 activity in supernatants (diluted 1:2) from various gluten-stimulated (middel panel) or *Mycobacterium tuberculosis*-stimulated (lower panel) (48 h) T cell clones (■) as indicated in relation to clonal control without relevant antigen (□), and reference values obtained with 0–250 ng/ml of rhIL-5 (■) (upper panel). Results are presented as mean ^{3}H-thymidine incorporation (ct/min) in triplicates tested in the same experiment (repeated in a reproducible manner at least twice). Insert shows autoradiogram after slot-blot hybridization with antisense DNA probe for IL-5 mRNA from three gluten-stimulated (2–18 h) T cell clones as indicated, compared with unstimulated control (0 h).

3 Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. J Exp Med 1989; **169**:345–50.

4 Sollid HM, Thorsby E. HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis. Gastroenterology 1993; **105**:910–22.

5 Halstensen TS, Scott H, Fausa O, Brandtzaeg O. Gluten stimulation of coeliac mucosa *in vitro* induces activation (CD25) of lamina propria $CD4^{+}$ T cells and macrophages but no crypt-cell hyperplasia. Scand J Immunol 1993; **38**:581–90.

6 Lundin KEA, Scott H, Hansen T *et al*. Gliadin-specific, HLA-DQ (α1*0501, β1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med 1993; **78**:187–96.

7 Lundin KEA, Scott H, Fausa O, Thorsby E, Sollid LM. T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8. Hum Immunol 1994; **41**:285–91.

8 Halstensen TS, Brandtzaeg P. Activated T lymphocytes in the celiac lesion: non-proliferative activation (CD25) of $CD4^{+}$ α/β cells in the lamina propria but proliferation (Ki-67) of α/β and γ/δ cells in the epithelium. Eur J Immunol; **23**:505–10.

9 Sikora K, Anand BS, Truelove SC, Ciclitira PJ, Offord RE. Stimulation of lymphocytes from patients with coeliac disease by a subfraction of gluten. Lancet 1976; 389–91.

10 Frew AJ, Bright S, Shewry PR, Munro A. Proliferative response of lymphocytes of normal individuals to wheat proteins (gliadin). Int Arch Allergy Appl Immunol 1980; **62**:162–7.

11 Scott H, Fausa O, Thorsby E. T-lymphocyte activation by a gluten fraction, glc-gli. Scand J Immunol 1983; **8**:185–91.

12 Gjertsen HA, Sollid LM, Ek J, Thornsby E, Lundin KEA. T cells from the peripheral blood of coeliac disease patients recognize gluten antigens when presented by HLA-DR, -DQ, or -DP molecules. Scand J Immunol 1994; **39**:567–74.

13 Gjertsen HA, Lunden KEA, Sollid LM, Eriksen JA, Thornsby E. T cells recognize a peptide derived from α-gliadin presented by the celiac disease-associated HLA-DQ (α1*0501, β1*0201) heterodimer. Hum Immunol 1994; **39**:243–52.

14 Jensen K, Sollid LM, Scott H, Paulsen G, Kett K, Thorsby E, Lundin KEA. Gliadin-specific T cell responses in peripheral blood of healthy individuals involve T cells restricted by the coeliac disease associated DQ2 heterodimer. Scand J Immunol 1995; **42**:166–70.

15 Nilsen EM, Lundin KEA, Krajci P, Scott H, Sollid LM, Brandtzaeg P. Gluten-specific, HLA-HQ-restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon-γ. Gut 1995; **37**:766–76.

16 Watson J. Continuous proliferation of murine antigen-specific helper T lymphocytes in culture. J Exp Med 1979; **150**:1510–19.

17 Helle M, Boeije L, Aarden LA. Functional discrimination between interleukin 6 and interleukin 1. Eur J Immunol 1988; **18**:1535–40.

18 Fattah D, Quint DJ, Proudfoot A, O'Malley R, Zanders ED, Champion BR. *In vitro* and *in vivo* studies with purified recombinant human interleukin 5. Cytokine 1990; **2**:112–21.

19 Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Methods 1986; **95**:99–105.

20 Danielpour D, Dart LL, Flanders KC, Roberts AB, Sporn MB. Immunodetection and quantitation of the two forms of transforming growth factor-beta (TGF-β1 and TGF-β2) secreted by cells in culture. J Cell Physiol 1989; **138**:79–86.

21 Kvale D, Krajci P, Brandtzaeg P. Expression and regulation of adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) in human intestinal epithelial cell lines. Scand J Immunol 1992; **35**:669–76.

22 Sollid LM, Gaudernack G, Markussen G, Kvale D, Brandtzaeg P, Thorsby E. Induction of various HLA class II molecules in a human colonic adenocarcinoma cell line. Scand J Immunol 1987; **25**:175–80.

23 Kvale D, Brandtzaeg P, Løvhaug D. Up-regulation of the expression of secretory component and HLA-molecules in a human colonic cell line by tumour necrosis factor-α and gamma interferon. Scand J Immunol 1988; **28**:351–7.

24 MacDonald RJ, Swift GH, Przybyla AE, Rutters WJ, Chirgwin JM. Isolation of RNA using guanidium salts. Methods Enzymol 1979; **152**:219–34.

25 Murray JS, Madri J, Tite J, Carding SR, Bottomly K. MHC control of $CD8^+$ T cell subset activation. J Exp Med 1989; **170**:2135–40.

26 Soloway P, Fish S, Passmore H, Gefter M, Coffe R, Manser T. Regulation of the immune response to peptide antigens: differential induction of immediate-type hypersensitivity and T cell proliferation due to changes in either peptide structure or major histocompatibility complex haplotype. J Exp Med 1991; **174**:847–58.

27 Mosmann TR, Coffman RL, TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; **7**:145–73.

28 Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994; **12**:227–57.

29 Yamamura M. Uyemura KI, Deans RJ, Weinburg K, Rea TH, Bloom BR, Modlin RL. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science 1991; **254**:277–9.

30 Clerci M, Hakim FT, Venzon DJ, Blatt S, Hendrix CH, Wynn TA, Shearer GM. Changes in interleukin-2 and interleukin-4 production in asymptomatic human immunodeficiency virus-seropositive individuals. J Clin Invest 1993; **91**:759–65.

31 Parronchi P, De Carli M, Manetti R *et al*. Aberrant interleukin (IL)-4 and IL-5 production *in vitro* by $CD4^+$ helper T cells from atopic subjects. Eur J Imunol 1992; **22**:1615–20.

32 Del Prete GF, De Carli M, D'Elios MM, Maestrelli P, Ricci M, Fabbri L, Romagnani S. Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. Eur J Immunol 1993; **23**:1445–9.