

Gluten specific suppressor T cell dysfunction in coeliac disease

G R CORAZZA, P SARCHIELLI, M LONDEI, M FRISONI, AND G GASBARRINI

From the I Department of Medical Pathology, S. Orsola University Hospital, University of Bologna, Bologna, Italy

SUMMARY A T lymphocyte direct migration inhibition factor test has been used to investigate the function of the specific suppressor T cell population controlling the immune response to gluten in coeliac disease. The test has been carried out in 21 adult coeliac patients, 22 Mantoux⁻ healthy controls and eight Mantoux⁺ donors using gluten fraction III and purified protein derivative, as antigens. All coeliacs, but two, were Mantoux⁻. When gluten fraction III was used a significant migration inhibition was observed in coeliac patients compared to controls; such migration inhibition was abrogated by coculturing in a 1:1 ratio coeliac T cells with T cells from controls or Mantoux⁺ donors. On the contrary, the addition to coeliac T cells of T lymphocytes from other coeliacs did not abolish migration inhibition to gluten. Pretreatment of normal T cells with mitomycin C prevented their abrogating activity on migration inhibition of coeliac T lymphocytes. When purified protein derivative was used as antigen a significant migration inhibition was observed in Mantoux⁺ donors compared with healthy subjects and such migration inhibition was abolished by co-culturing T cells from Mantoux⁺ donors with those from Mantoux⁻ controls and coeliac patients. Our results show that coeliac T cells, while retaining their ability to suppress the immune response to purified protein derivative, cannot suppress the immune response to gluten and are consistent with the hypothesis that a gluten specific suppressor T cell dysfunction, rather than a generalised T lymphocyte defect, may play a role in the pathogenesis of coeliac disease.

Coeliac disease is characterised by small intestinal mucosal injury caused by the ingestion of dietary gluten in susceptible individuals.¹ Evidence, such as the derangement in the usual proportions of jejunal intraepithelial lymphocytes and enterocytes² and the humoral and cell mediated hyperresponsiveness to gluten,³⁻⁶ support the importance of the immune system in the pathogenesis of coeliac disease.

A defect in the mechanisms of immune regulation has been proposed as the fundamental abnormality in the afferent limb of the immune response to gluten,⁷ however, previous studies^{8,9} have failed to show any imbalance in the OKT4/OKT8 T cell ratio in the peripheral blood of untreated and treated coeliac patients. The unaltered balance of T cell subsets cannot exclude the presence of a gluten specific defect of suppressor function that, due to its

antigenic restriction, may be such not to affect the global inducer/suppressor T cell ratio.

Recently, a defective antigen specific suppressor function has been demonstrated, using a T lymphocyte migration inhibition system both in autoimmune thyroid disease¹⁰ and in autoimmune chronic active hepatitis.¹¹

On the basis of these studies, the aim of the present work has been to verify whether the addition of normal T lymphocytes, but not of lymphocytes from other coeliac patients, can revert gluten induced migration inhibition of coeliac T lymphocytes, that is to say whether evidence can be found of an antigen specific suppressor dysfunction in coeliac disease.

Methods

PATIENTS

Twenty one adult coeliac patients — 4 untreated and 17 on a gluten-free diet-, eight Mantoux⁺ subjects and 22 healthy volunteers were studied.

The diagnosis of coeliac disease was based on the

Address for correspondence: G R Corazza, MD, I Patologia Medica, Nuove Patologie, Policlinico S. Orsola, Via Massarenti 9, 40138 Bologna, Italy.

presence of the typical jejunal mucosal abnormalities with histological improvement after gluten withdrawal. In the four untreated coeliac patients, although a second biopsy has not yet been done, a clinical response to gluten withdrawal, that started after the present study was over, has been already observed. Only two Mantoux⁺ coeliacs were included in the study and all coeliacs but one showed a normal cell mediated immunity as assessed by a new Multitest system (Institute Mérieux, Lyon, France).¹²

Mantoux⁺ subjects and healthy volunteers were defined by the presence or absence, respectively, of an induration greater than 10 mm in diameter 48 h after application of 1 IU of purified protein derivative intradermally.

ISOLATION OF T LYMPHOCYTES FROM PERIPHERAL BLOOD

Mononuclear cells from approximately 100 ml of heparinised venous blood were isolated by the standard Ficoll density gradient centrifugation.¹³ These cells, suspended in 10 ml RPMI medium, were mixed with an equal volume of 1% neuraminidase-treated red blood cells, incubated at 37°C for 15 min, then centrifuged at 200×g for five min and reincubated at 4°C for 90 min. Rosetted cells (T lymphocytes) were isolated from non-rosetted cells by the Ficoll-Hypaque gradient technique. The pellet containing the E-rosetted T lymphocytes was treated with 0.84% ammonium chloride to remove by lysis the adherent sheep red blood cells and washed three times before use in the migration inhibition factor test.

T LYMPHOCYTE MIGRATION INHIBITION FACTOR TEST (MIF TEST)

The assay was done according to Bullen and Losowsky.⁵ Isolated T lymphocytes, resuspended in RPMI medium at a concentration of 30×10⁶ cells/ml were filled into 20 µl microcapillary tubes. When cell mixtures were studied they were adjusted to the desired concentration. Migration inhibition factor test was always done in triplicate and the migration index was calculated as mean area of migration in the presence of antigen divided by mean area of migration in the absence of antigen. The antigens used were gluten fraction III (GFIII), prepared from BDH gluten according to the method of Frazer *et al.*,¹⁴ and purified protein derivative (PPD; Weybridge, Surrey, UK). On the basis of previous studies^{5 15} and of preliminary experiments of ours GFIII and PPD were used at the final concentration of 1 mg/ml and 100 µg/ml, respectively.

In some experiments T lymphocytes, from either coeliac patients or healthy controls, were suspended in medium (2×10⁶/ml) and preincubated with mitomycin C(20 µg/ml) for 30 min at 37°C according to Okita *et al.*¹⁰ T lymphocytes were then washed three times with control medium and used in the direct MIF test.

HLA-DR TYPING

This was done in all coeliac patients according to the technique described by Terasaki *et al.*¹⁶

STATISTICAL ANALYSIS

The results were compared using the non-parametric Wilcoxon's rank sum test.

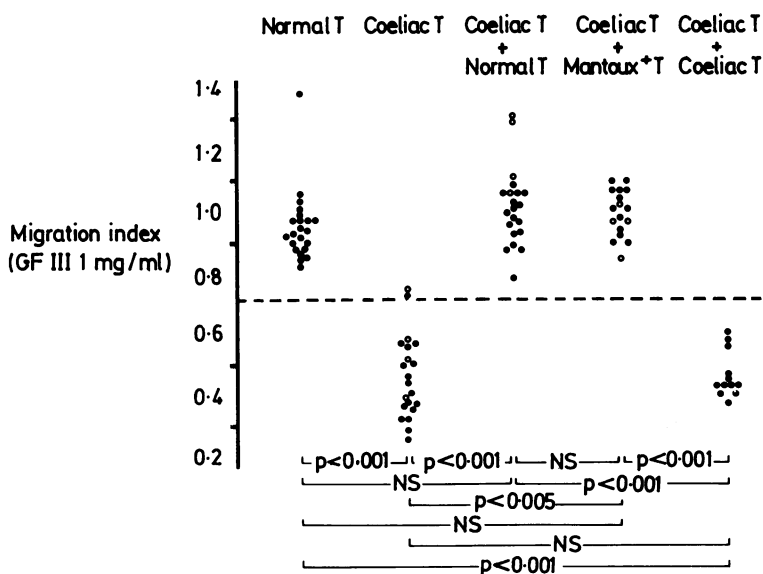


Fig. 1 Migration indices to GFIII of T lymphocytes from normal subjects, coeliac patients (● treated, ○ untreated), coeliac patients plus normal subjects, coeliac patients plus Mantoux⁺ donors, coeliac patients plus other coeliac patients. In coculture experiments cells were used in a 1:1 ratio. Broken line represents the upper limit of the sensitised area.

Results

Migration indices to GFIII are shown in Figure 1. Values below two SD of the mean control value were taken to indicate sensitisation to GFIII. T lymphocyte migration in coeliac patients (\bar{x} 0.46 ± 0.13) was significantly inhibited as compared with normal subjects (\bar{x} 0.95 ± 0.11), and all coeliacs but two showed migration indices in the sensitised range. When T lymphocytes from normal or Mantoux⁺ subjects were mixed with T lymphocytes from coeliac patients in a 1:1 ratio, however, migration inhibition was significantly abolished – that is, migration indices (\bar{x} 1.01 ± 0.12 and 0.99 ± 0.07 , respectively) fell in the non-sensitised range and did not differ significantly from those of normal T lymphocytes alone. On the contrary, when T cells from coeliac patients were mixed with T cells from other coeliac patients in the same 1:1 ratio, migration indices (\bar{x} 0.43 ± 0.15) fell in the sensitised range and did not differ significantly from those of coeliac T lymphocytes alone.

Migration indices to PPD are shown in Figure 2. T lymphocyte migration in Mantoux⁺ subjects (\bar{x} 0.53 ± 0.11) was significantly inhibited as compared with normal subjects (\bar{x} 0.93 ± 0.09), and all but one Mantoux⁺ subjects showed migration indices in the sensitised range. As observed in coeliac patients using GFIII as antigen, however when T lymphocytes from Mantoux⁺ subjects were mixed with T lymphocytes from normal subjects or coeliac patients in a 1:1 ratio, migration indices (\bar{x} 1.10 ± 0.17 and

0.95 ± 0.20 , respectively) were significantly higher than those of Mantoux⁺ T lymphocytes alone and did not differ significantly from those of normal T lymphocytes. Only in two coculture experiments migration inhibition was not abolished: in both cases Mantoux⁺ T lymphocytes had been mixed with T lymphocytes from the two Mantoux⁺ coeliac patients.

Figure 3 shows the results obtained coculturing in different proportions T cells from three coeliac patients with those from three normal subjects, in the presence of GFIII (1 mg/ml). As expected migration inhibition was observed in the 10:0 ratio (coeliac T lymphocytes alone), but not in the 0:10 ratio (normal T lymphocytes alone). Migration inhibition of coeliac T lymphocytes was abolished by co-culture with normal T lymphocytes, not only in the 5:5 ratio, the same used throughout our work, but even in the 9:1 ratio – that is, 1 part of normal T lymphocytes is able to prevent migration inhibition of 9 parts of coeliac T lymphocytes.

Figure 4 shows that pretreatment with mitomycin C does not affect either the migration of normal T lymphocytes, or the inhibited migration of coeliac T lymphocytes to GFIII. On the contrary, the abolition of migration inhibition seen when coeliac T lymphocytes are cocultured with normal T lymphocytes is prevented when the latter are pretreated with mitomycin C. For 10 experiments in which coeliac T lymphocytes were cocultured with T lymphocytes from other coeliacs, the HLA-DR pattern of the 20 patients involved was known. The

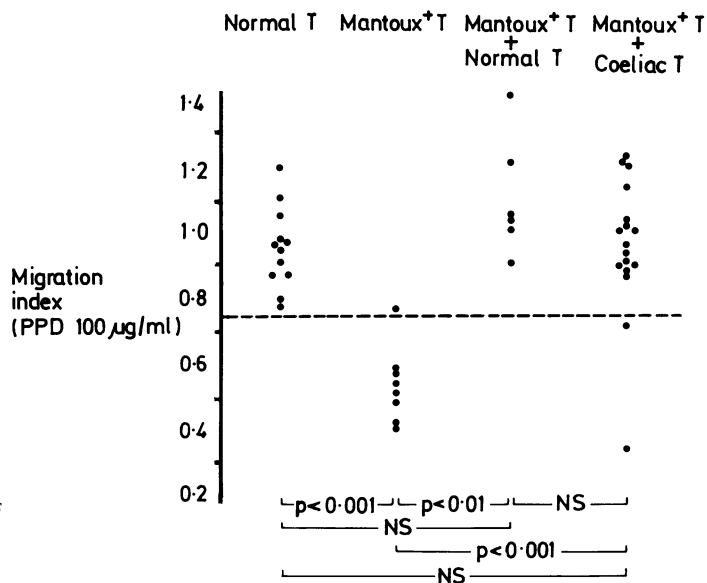


Fig. 2 Migration indices to PPD of T lymphocytes from normal subjects, Mantoux⁺ donors, Mantoux⁺ donors plus normal subjects, Mantoux⁺ donors plus coeliac patients. In coculture experiments cells were used in a 1:1 ratio. Broken line represents the upper limit of the sensitised area.

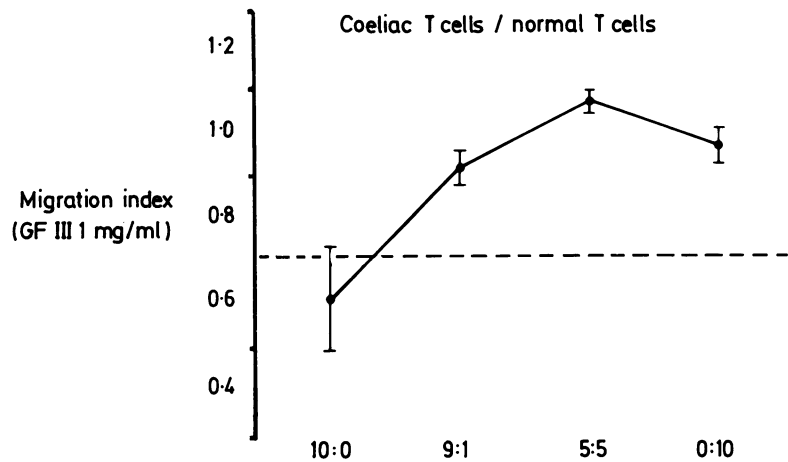


Fig. 3 Migration indices ($\bar{x} \pm SD$) to GFIII obtained coculturing T lymphocytes from three coeliac patients with those from three normal subjects in various ratios. Broken line represents the upper limit of the sensitised area.

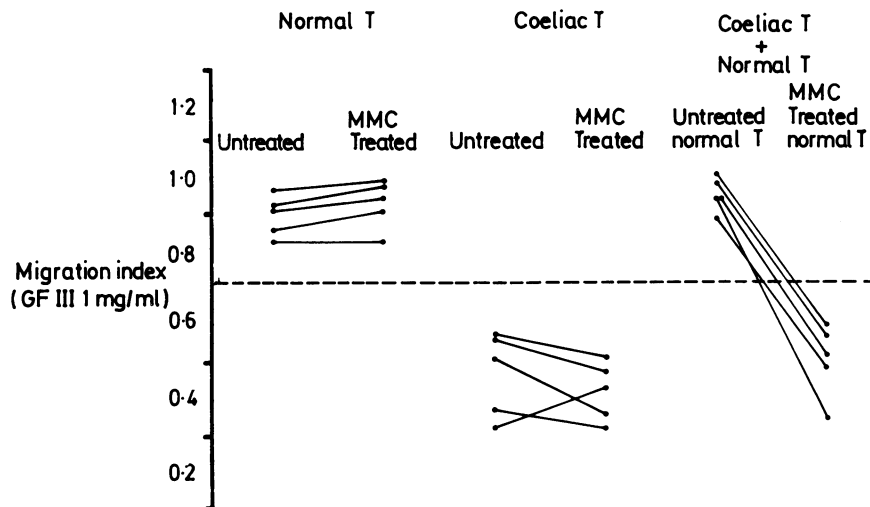


Fig. 4 Effect of pretreatment with mitomycin C(MMC) on the migration indices to GFIII of T lymphocytes from five normal subjects, five coeliac patients and five normal subjects cocultured with T lymphocytes from five coeliac patients. Broken line represents the upper limit of the sensitised area.

mean migration index of the five HLA-DR identical pairs (0.46 ± 0.08) was very close to that of the five HLA-DR dissimilar pairs (0.47 ± 0.09).

Discussion

In the MIF test sensitised lymphocytes were held to be the lymphokine producing cells, but not the cells inhibited by antigen.¹⁷ More recently, however, migration inhibition of purified peripheral T lymphocytes by specific antigen and lymphokines has been shown¹⁸⁻²⁰ and the response of lymphocytes alone has been reported to be greater than that of

leucocytes or mononuclear cells.¹⁸ In our work purified preparations of T lymphocytes from coeliac patients have been incubated with GFIII and this may account for the greater sensitivity of the MIF test in coeliac disease observed in this work compared with other studies,^{5, 21} in which mixed leucocytes from coeliac patients had been incubated with GFIII.

Earlier studies on concanavalin A activated suppressor function in coeliac disease produced contrasting results.²²⁻²⁴ Thus it seems necessary to employ a specific antigen to investigate immune regulation in coeliac disease, a condition assumed to

be the result of an abnormal immune response to gluten.

Our results using a T lymphocyte direct migration inhibition test show that inhibition of migration of T cells from coeliac patients in response to GFIII is abrogated by coculture with T lymphocytes from non-coeliac subjects but not with T lymphocytes from other coeliac patients. Otherwise, with the exception of two experiments in which T cells from two coeliac patients who were also Mantoux⁺ were used, coeliac T lymphocytes, as well as normal T lymphocytes, do abrogate migration inhibition to PPD of T cells from Mantoux⁺ donors. This observation is in keeping with the hypothesis that migration inhibition of coeliac T lymphocytes to GFIII arises from a defect of suppressor T cell function which allows helper T cells to produce MIF.^{25, 26} The addition of non-coeliac T lymphocytes abrogates MIF production that, on the contrary, is not affected by the addition of T lymphocytes from other coeliac patients which are unable to abrogate the immune response to gluten. Coeliac T cells retain their ability to suppress the immune response of PPD sensitised T lymphocytes, supporting the view that in coeliac disease the defect of suppressor function is antigen specific.

The abolition of MIF production by gluten sensitised coeliac T cells brought forth by T lymphocytes of healthy or Mantoux⁺ subjects cannot be ascribed to a simple dilution effect as it can be observed not only in a 1:1 ratio, but also when the ratio coeliac T: normal T is 9:1. On the other hand, the possibility that our data are the result of a mixed lymphocyte reaction is disproved by a number of evidences, already considered in the work of Okita, Row, and Volpe.¹⁰ An allogeneic mixed lymphocyte reaction has been shown to promote and not to impair migration inhibition,²⁷ not only, but this type of reaction would not explain the different results observed when coeliac T lymphocytes are cocultured, in the presence of gluten, with other coeliac T lymphocytes or with T lymphocytes from healthy or Mantoux⁺ donors. Our view that the gluten specific defect of coeliac T cell suppressor function is corrected by normal T lymphocytes is supported by the effect on cocultures of the pretreatment of normal T lymphocytes with mitomycin C, an agent that impairs selectively T cell suppressor function, but not helper function.²⁸ In fact, in our study the pretreatment of coeliac T lymphocytes with mitomycin C did not affect migration inhibition whereas the pretreatment of normal T lymphocytes prevented the abrogation of migration inhibition, thus suggesting that the effect of normal T cells on coeliac T cells is one of allosuppression. Certainly an allogeneic mixed lymphocyte reaction cannot be

held responsible for the results obtained coculturing T lymphocytes from different coeliac patients. In fact, it is accepted that an allogeneic mixed lymphocyte reaction is stimulated by differences in the pattern of HLA-DR antigens,²⁹ whereas in our study migration indices of paired HLA-DR dissimilar coeliacs did not differ from those of paired HLA-DR identical coeliacs. Finally, it is unlikely that migration inhibition observed in cocultures of T lymphocytes from different coeliac patients is the result of an independent stimulation by gluten of the two cell preparations: in fact migration indices very low in the sensitised range have also been observed in those three experiments that used T lymphocytes from the two patients no longer sensitised to gluten.

The gluten specific defect of suppressor function here reported, further supports the view that coeliac disease results from abnormal immunological phenomena. Such a defect appears to be primitive, because it is present in coeliacs on a gluten free diet for many years, and seems to be pathogenetically important.

It is known that the exposure to an antigen by the oral route is followed by a state of antigen specific systemic tolerance.^{30, 31} Such a tolerance, as a consequence of antigen feeding, has been shown to be associated with the appearance of antigen specific suppressor T cells induced locally in the gut associated lymphoid tissue that subsequently migrate to extra-intestinal sites.³²⁻³⁴ In coeliac disease this equilibrium may be abrogated due to the gluten-specific defect of suppressor function and such a defect fits well with other immunological abnormalities already described in coeliac patients. HLA determinants contribute to confer susceptibility to coeliac disease³⁵ and recently existence of HLA-linked immune-suppression genes has been suggested,³⁶ therefore it is possible to speculate that the primitive gluten specific defect of suppressor function depends on a gene in linkage disequilibrium with one of the HLA alleles associated with coeliac disease. As a consequence patients with coeliac disease present in peripheral blood and jejunal mucosa both humoral^{3, 4} and cell mediated^{5, 6} hyperresponsiveness to gluten. Coeliac enterocytes might become target of such a response because of the binding of gluten to their surfaces¹ and accordingly a glycopeptide component of gluten has been shown to bind to intestinal cells membranes of coeliac patients more than to those of healthy subjects.³⁷ Moreover, it is conceivable that an increase in the number of gluten binding sites is not specific of coeliac disease but may also occur in intestinal disorders other than coeliac disease because of an increased number of immature enterocytes. In these situations the presence of

gluten receptors may bring to transient gluten susceptibility but not to coeliac disease.³⁸ For this to develop the contribution of some major abnormalities of the immune response to gluten seems mandatory. We suggest that this abnormality is represented by a gluten specific suppressor cell dysfunction.

The authors thank Marinella Cenci and Donatella Bastia for technical assistance and Dr Sandro Vento for helpful discussion.

References

- Falchuk ZM. Update on gluten-sensitive enteropathy. *Am J Med* 1979; **67**: 1085-96.
- Corazza GR, Frazzoni M, Gasbarrini G. Jejunal intraepithelial lymphocytes in coeliac disease: are they increased or decreased? *Gut* 1984; **25**: 158-62.
- Taylor KB, Thomson DL, Truelove SC, Wright R. An immunological study of coeliac disease and idiopathic steatorrhoea. Serological reactions to gluten and milk products. *Br Med J* 1961; **2**: 1727-31.
- Falchuk ZM, Strober W. Gluten-sensitive enteropathy: synthesis of anti gliadin antibody in vitro. *Gut* 1974; **15**: 947-52.
- Bullen AW, Losowsky MS. Cell-mediated immunity to gluten fraction III in coeliac disease. *Gut* 1978; **19**: 126-31.
- Ferguson A, MacDonald TT, McClure JP, Holden RJ. Cell-mediated immunity to gliadin within the small-intestinal mucosa in coeliac disease. *Lancet* 1975; **1**: 895-7.
- Strober W. An immunological theory of gluten-sensitive enteropathy. In: McNicholl B, McCarthy CF, Fottrell PF, eds. *Perspectives in coeliac disease*. Lancaster: MTP Press, 1978: 169-82.
- Corazza GR, Tabacchi P, Frisoni M, Londei M, Bastia D, Gasbarrini G. T lymphocyte subsets in adult coeliac disease. *Clin Sci* 1983; **65**: 89-90.
- Selby WS, Janossy G, Bofill M, Jewell DP. Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. *Clin Exp Immunol* 1983; **52**: 219-28.
- Okita N, Row VV, Volpè R. Suppressor T-lymphocyte deficiency in Graves' disease and Hashimoto's thyroiditis. *J Clin Endocrinol Metab* 1981; **52**: 528-33.
- Vento S, Hegarty JE, Bottazzo GF, Macchia E, Williams R, Eddleston ALWF. Antigen specific suppressor cell function in autoimmune chronic active hepatitis. *Lancet* 1984; **1**: 1200-4.
- Bjorkholm M, Holm G, Mellstedt H. No effect of delayed cutaneous hypersensitivity testing (Multitest) on blood lymphocyte counts and functions. *Acta Med Scand* 1983; **214**: 399-401.
- Boyum A. Separation of leucocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968; **21**: 77-109.
- Frazer AC, Fletcher RF, Ross CAC, Shaw B, Sammons HG, Schneider R. Gluten-induced enteropathy. The effect of partially digested gluten. *Lancet* 1959; **2**: 252-5.
- Bullen AW, Losowsky MS. Comparison of a leucocyte adherence test with the leucocyte migration inhibition test and skin reactivity to PPD. *Clin Exp Immunol* 1978; **31**: 408-13.
- Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A,B,C and DR antigens. *Am J Clin Pathol* 1978; **69**: 103-20.
- Clausen JE. Polymorphonuclear leucocytes in the specific antigen induced inhibition of the *in vitro* migration of human peripheral leucocytes. *Acta Med Scand* 1970; **188**: 59-64.
- Kowalczyk D, Zembala M. Migration inhibition of T-lymphocytes from human peripheral blood by specific antigen and lymphokines. *Clin Exp Immunol* 1978; **32**: 333-8.
- Okita N, Kidd A, Row VV, Volpè R. Sensitization of T-lymphocytes in Graves' and Hashimoto's diseases. *J Clin Endocrinol Metab* 1980; **51**: 316-20.
- Okita N, Topliss D, Lewis M, Row VV, Volpè R. T lymphocyte sensitization in Graves' and Hashimoto's diseases confirmed by an indirect migration inhibition factor test. *J Clin Endocrinol Metab* 1981; **52**: 523-7.
- Corazza GR, Rawcliffe PM, Frisoni M, Sarchielli P, Londei M, Campieri M, Lazzari R, Gasbarrini G. Specificity of leucocyte migration inhibition test in coeliac disease. A reassessment using different gluten subfractions. *Clin Exp Immunol* 1985; **60**: 117-22.
- Robertson DAF, Bullen A, Field H, Simpson FG, Losowsky MS. Suppressor cell activity, splenic function and HLA B8 status in man. *J Clin Lab Immunol* 1982; **9**: 133-8.
- O'Farrelly C, McKeever V, Feighery C, Weiz DG. Increased concanavalin A induced suppression in treated and untreated coeliac disease. *Gut* 1984; **25**: 644-8.
- Pignata C, Troncone R, Monaco G, Ciriaco M, Farris E, Carminati G, Auricchio S. Impaired suppressor activity in children affected by coeliac disease. *Gut* 1985; **26**: 285-90.
- Adelman NE, Ksiazek J, Yoshida T, Cohen S. Lymphoid sources of murine migration inhibition factor. *J Immunol* 1980; **124**: 825-30.
- Fox RA, Rajaraman K. A link between helper and suppressor factors and the lymphokines migration inhibition factor and migration stimulation factor. *Cell Immunol* 1981; **59**: 448-54.
- Cochrum KC, Salvatierra O, Cullen BL, Perkins HA, Hanes DM, Fudenberg HH. Leucocyte migration inhibitory factor (LIF) as an indicator of mixed lymphocyte culture (MLC) reactivity. *Transplant Proc* 1977; **9**: 97-101.
- Swain DL, Dutton RW. Negative allogeneic effects in vitro. I. Allogeneic T cells markedly suppress the secondary antibody-forming cell response. *J Immunol* 1977; **118**: 2262-8.
- Smith JB. Stimulation of autologous and allogeneic human T cells by B cells occurs through B-cell antigen systems. *Cell Immunol* 1978; **36**: 203-9.

- 30 Chase MW. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. *Proc Soc Exp Biol Med (NY)* 1946; **61**: 257-9.
- 31 Thomas HC, Parrott DMV. The induction of tolerance to a soluble protein antigen by oral administration. *Immunology* 1974; **27**: 631-9.
- 32 Ngan J, Kind LS. Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin. *J Immunol* 1978; **120**: 861-5.
- 33 Mattingly JA, Waksman BH. Immunologic suppression after oral administration of antigen. I. Specific suppressor cells formed in rat Peyer's patches after oral administration of sheep erythrocytes and their systemic migration. *J Immunol* 1978; **121**: 1878-83.
- 34 Miller SD, Hanson DG. Inhibition of specific immune responses by feeding protein antigens. IV. Evidence for tolerance and specific active suppression of cell mediated immune responses to ovalbumin. *J Immunol* 1979; **123**: 2344-50.
- 35 Corazza GR, Tabacchi P, Frisoni M, Prati C, Gasbarrini G. DR and non-DR Ia allotypes are associated with susceptibility to coeliac disease. *Gut* 1985; **26**: 1210-13.
- 36 Sasazuki T, Nishimura Y, Muto M, Ohta N. HLA-linked genes controlling immune response and disease susceptibility. *Immunological Rev* 1983; **70**: 51-75.
- 37 Douglas AP. The binding of a glycopeptide component of wheat gluten to intestinal mucosa of normal and coeliac human subjects. *Clin Chim Acta* 1976; **73**: 357-61.
- 38 Weiser MM, Douglas AP. An alternative mechanism for gluten toxicity in coeliac disease. *Lancet* 1976; **1**: 567-69.