Human Leukocyte Antigen–DQ2 Homozygosity and the Development of Refractory Celiac Disease and Enteropathy-Associated T-Cell Lymphoma

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Background & Aims: **Celiac disease (CD) is a common gluten-sensitive enteropathy associated with human leukocyte antigen (HLA)-DQ2 and HLA-DQ8. The aim of this study was to determine if a particular HLA-DQ subtype predisposes to complications such as refractory CD with (RCD II) or without aberrant T cells (RCD I), and enteropathy-associated T-cell lymphomas (EATL).** ***Methods:*** **Molecular HLA-DQ typing was performed on 43 RCD I, 43 RCD II, and 30 EATL patients, and compared with age-matched groups of 121 patients with histologically defined uncomplicated CD and 183 healthy controls. All individuals were Dutch Caucasians and were at least 21 years of age.** ***Results:*** **HLA-DQ2 was present in 79% of RCD I, 97.7% of RCD II, and 96.6% of EATL patients. The differences were significant when compared with 28.9% in controls but not with 91.7% in uncomplicated CD. Homozygosity for HLA-DQ2 was observed in 25.5% of RCD I, 44.1% of RCD II, and 53.3% of EATL patients vs 20.7% of uncomplicated CD patients and 2.1% of controls. HLA-DQ8 was present in 10.7% of CD, 16.2% of RCD I, 9.3% of RCD II, and 6.6% of EATL patients vs 20.2% of controls.** ***Conclusions:*** **Homozygosity for HLA-DQ2 is associated with RCD II and EATL. Early identification of HLA-DQ2 homozygous CD patients may help to recognize CD patients at risk for developing these severe complications.**

Celiac disease (CD) is a common gluten-sensitive enteropathy affecting 1 in 150–300 individuals worldwide.[1,2] CD is associated strongly with the class II human leukocyte antigen (HLA)-DQ2 heterodimer encoded by the DQA1*0501 and DQB1*02 alleles. The DQ2 glycoprotein is present in 90%–95% of Caucasian CD patients.[3,4] The majority of DQ2-negative CD patients are positive for the haplotype DQA1*03-DQB1*0302 (HLA-DQ8).[5–7] A small number of CD patients lacking these heterodimers have either DQA1*05 or DQB1*02 alone.[8] CD-associated HLA-DQ molecules bind and present gluten peptides to antigen-specific T cells. These HLA-DQ–peptide complexes induce inflammatory T-cell responses in the small intestine with villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis.[4,9] HLA-DQ2 homozygous antigen-presenting cells induce higher T-cell proliferation and cytokine secretion than HLA-DQ2/non–DQ2 heterozygous antigen-presenting cells.[9] This may explain the strongly increased risk for disease development in HLA-DQ2 homozygous individuals.[10–13]

In a small percentage of patients serious complications develop. CD patients may be regarded as suffering from refractory CD (RCD) when symptoms persist or recur after a former good response despite strict adherence to a gluten-free diet. When normal expression of T-cell antigens and polyclonal T-cell receptor (TCR) gene rearrangement occur (RCD I) the prognosis is less dismal than when an aberrant clonal intraepithelial lymphocytes (IEL) population and/or loss of antigens on IELs is present (RCD II). These patients have a high risk for developing intestinal lymphoma.[14–17] Patients with refractory CD are at a greater risk for developing malignancy.[18] EATL has histologic and immunohistochemical features of large- or medium-size T-cell proliferation expressing a CD3+ CD8+/− and CD103+ phenotype. The majority of these lymphomas present as CD3+ CD8− CD30+ large-cell lymphoma, however, small-cell lymphomas, often CD3+ CD8+ CD30−, may occur.[19]

We have investigated whether a distinct HLA-DQ subgroup represents a risk factor for the development of refractory disease and the development of EATL.

Abbreviations used in this paper: CD, celiac disease; CI, confidence interval; HLA, human leukocyte antigen; IEL, intraepithelial lymphocytes; OR, odds ratio; RCD, refractory CD; TCR, T-cell receptor.

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Table 1. Age and Sex Distribution of the Patients With Histologically Defined Uncomplicated CD (Marsh III), RCD I, RCD II, EATL, and Controls

	Mean age at diagnosis of CD, *y* (range)	Mean age at diagnosis of RCD I, RCD II, and EATL, *y* (range)	Male:female ratio
Uncomplicated CD (n = 121)	45.9 (22–75)	—	24:97
RCD I (n = 43)	47 (21–75)	49 (23–86)	12:31
RCD II (n = 43)	57 (40–69)	59 (47–88)	19:24
EATL (n = 30)	59 (46–69)	61.5 (52–79)	16:14
Controls (n = 183)	38.7 (24–89) at participation	—	85:98

Materials and Methods

Patients

Forty-three patients with RCD I (12 men, 31 women; mean age at diagnosis, 49 y; range, 23–86 y), 43 patients with RCD II (19 men, 24 women; mean age at diagnosis, 59 y; range, 47–88 y), and 30 patients with EATL (16 men, 14 women; mean age at diagnosis, 61.5 y; range, 52–79 y) were studied. Patients were referred to the Rijnstate Hospital or the VU University Medical Centre, both of which are tertiary referral centers for CD, and were recruited from all provinces in The Netherlands from 1992 to 2003. The patients with RCD I and II were followed-up over a mean period of 5 years (range, 2–12 y) for evidence of transition to a more severe state (ie, the transition from RCD I to RCD II and /or EATL, and from RCD II to EATL).

For comparison we used data on 121 unrelated and uncomplicated Dutch Caucasian CD patients (24 men, 97 women; mean age at diagnosis, 45.9 y; range, 22–75 y) selected by age to match the age groups under study. All these patients had villous atrophy (Marsh type III) on a normal gluten-containing diet and responded with histologic and clinical improvement to withdrawal of gluten from the diet.[20] Table 1 shows the demographic characteristics of these patients and the age at diagnosis of both CD and the complicated state (RCD and EATL).

A group of 183 unrelated healthy Dutch Caucasians (85 men, 98 women; mean age at participation, 38.7 y; range, 24–89), previously typed for HLA-DQ served as controls.[20]

In complicated CD, possible underlying diseases (except EATL) such as bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple's disease, hypogammaglobulinemia, eosinophilic enteritis, and inflammatory bowel disease were excluded. In addition to endoscopic and histopathologic evaluation, all patients with complicated CD underwent clinical, laboratory, and radiologic assessment including intraepithelial lymphocyte phenotyping for signs of monoclonality; small-bowel radiograph and/or magnetic resonance imaging; serologic results for antigliadin, antiendomysium, and tissue transglutaminase levels; thyroid function tests; stool examination for giardia and other parasites; human immunodeficiency virus serology; and a dual-energy x-ray absorptiometry scan as part of a routine work-up.[1,17] When indicated, computed tomography scans of the abdomen, positron emission tomography, video capsule endoscopy, and/or double-balloon enteroscopy were performed.

Criteria for Diagnosis

The histology in the gluten-sensitive spectrum was categorized according to the modified Marsh criteria adapted by the working group of the 2001 United European Gastroenterology Week in Amsterdam.[21] The diagnosis of CD was confirmed by histologic examination with a documented histologic response to gluten withdrawal.[21] Patients with CD were considered to be refractory when symptoms of malabsorption owing to villous atrophy persisted or recurred after a former good response despite strict adherence to a gluten-free diet. The diagnosis of RCD was established as type I when no aberrant T cells were present in intestinal biopsy specimens and as type II when aberrant T cells were detected by immunophenotyping using flow-cytometric analysis or immunohistology of the intestinal mucosa.[17,21] In RCD I the IEL phenotype is normal with the expression of surface CD3, CD8, and TCR-β. In RCD II the IELs have normal cytologic features but they show an abnormal IEL phenotype with the expression of intracytoplasmic CD3ϵ, surface CD103, and the lack of classic surface T-cell markers such as CD4, CD8, and TCR-αβ.[18] The diagnosis of EATL was established according to the World Health Organization classification of tumors of hematopoietic and lymphoid tissues.[19,22,23] The immunohistochemical features of EATL are evidence of large- or medium-size T-cell proliferation expressing CD3+, CD8+/−, and CD103+. The majority are CD3+, CD8−, and CD30+ large-cell lymphomas; however, small-cell lymphomas often are CD3+, CD8+, and CD30−.[19]

Human Leukocyte Antigen–DQ Typing

Whole blood was obtained for typing of HLA-DQA1* and DQB1* alleles, performed with a combined single-stranded conformation polymorphism/heteroduplex method by a semiautomated electrophoresis and gel staining method on the Phastsystem (Amersham-Pharmacia-Biotech, Uppsala, Sweden).[20,24]

Statistical Analysis

Statistical data were analyzed by the Student *t* test, and results were considered statistically significant at a *P* value of less than .05. Odds ratios (ORs) and their 95% confidence intervals (CIs) were used to assess the significance of association between the dosage of haplotypes HLA-DQ2 and/or DQ8 and the risk for having RCD I, RCD II, and EATL.

Results

Table 1 shows the age and sex distribution of the patients with histologically defined uncomplicated CD (Marsh III), RCD I, RCD II, EATL, and controls.