the exposure over the previous hours (15) were collected and stored at −20 °C until analysis (14). Creatinine was assessed in urine according to a kinetic method adapted from the Jaffe method (16). Urinary cotinine results, calculated taking into account the fivefold urine concentration, were expressed with reference to creatinine values. Results were compared using the Student t-test.

Urinary cotinine values in children exposed to ETS (14.1 ± 11.9 mg/mol of creatinine) were significantly higher (P < 0.001) than those in nonexposed children (5.1 ± 5.4 mg/mol of creatinine). The proposed cotinine EIA thus enables nonsmokers exposed to ETS to be differentiated from those not exposed. The technique is simple, rapid, can be used in any laboratory having an automated analyzer (open system), and can be applied to large-scale studies.

This method can be recommended in epidemiological studies as a control for smoking, an important confounding factor often poorly described in self-administered questionnaires (17).

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References


IgG Autoantibodies against Tissue Transglutaminase in Relation to Antinuclear Antibodies

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The enzyme transglutaminase (tTg; EC 2.3.2.13) catalyzes, among others, the formation of e-(γ-glutamyl)-lysine bonds between substrate proteins, leading to cross-linked protein polymers (1). The enzyme is synthesized by a broad spectrum of cell types and is widely distributed in human organs (2). Induction and activation of tTg is part of the apoptotic cascade and plays an effector role in this process (3, 4). The enzyme is present in preapoptotic cells and enables the production of a highly cross-linked protein scaffold in apoptotic cells, joining cytoplasmic and membrane proteins and thus maintaining cellular integrity during the formation of apoptotic bodies. This cross-linking of proteins stabilizes the apoptotic bodies and limits the leakage of intracellular components into the extracellular space (1, 5).

Recently, tissue tTg has been shown to be the key autoantigen of the so-called anti-endomysium antibodies (IgA type), which are diagnostic for celiac disease (6). IgG antibodies against tTg (IgG anti-tTg) can be found in other autoimmune diseases in humans (7) and animals (1). There is ample evidence of the relationship between autoimmune disease and dysbalanced apoptosis, but the mechanisms remain hypothetical (8, 9). It has been suggested that a deficiency of the apoptotic removal of autoreactive immune cells breaks the self-tolerance (10). A second hypothesis is that the clearance of apoptotic bodies is deficient, with the result that their contents come into contact with the immune system, leading to autoimmunity as well (11–13). Accordingly, the demonstration of autoantibodies against tTg may be of great interest in the diagnosis and follow-up of autoimmune diseases and may throw a new light on their etiology. We therefore developed a time-resolved fluoroimmunoassay (TRFIA) to measure IgG anti-tTg.

The TRFIA method was as follows. tTg from guinea pig liver (Sigma) was used as antigen. F(ab')2 fragments from antibodies raised in rabbits against human IgG (Dako A/S) labeled with Eu³⁺, using the reagent and procedure from Wallac Oy, served as the tracer. Samples (diluted
matoid factor (15), not turbidimetry (Tina-quant®; Roche). Antinuclear antibodies were detected by immunofluorescence on Hep-2000® cells (Biomedical Diagnostics) and identified by immunoblotting using the Innolia® ANA reagent set (Innogenetics).

Sera were selected from our serum bank (in which they were stored at −80 °C for periods of 1 week to 8 years) on the basis of their antibody pattern and clinical diagnosis: (a) 30 sera from patients with systemic lupus erythematosus (SLE; n = 15) with increased anti-dsDNA; (b) serum samples with different anti-nuclear antibodies [SSA and SSA/SSB (Ro, La; n = 13), Sm/RNP and RNP (n = 21)], anti-nucleolar antibodies (immunofluorescence; n = 7), anti-histidyl-tRNA synthetase (Jo-1; n = 5), anti-centromere protein B (Cenp-B; n = 14), or anti-topoisomerase I (Scl-70; n = 7); and (c) serum samples containing other autoantibodies, such as IgM rheumatoid factor (n = 30), anti-proteinase 3 (n = 14), and IgA anti-gliadin (n = 9). The samples contained in general not more than one detectable antibody (in addition to anti-βTG) other than the one mentioned. All patients described here suffered from chronic inflammation. To exclude the possibility that IgG anti-βTG is an aspecific marker for this condition, we tested sera (n = 14) with increased C-reactive protein concentrations. We found IgG anti-βTG concentrations comparable to those in control subjects (Fig. 1). The procedures followed were in accordance with the policies of the Ethical Review Board of the hospital group.

High concentrations of IgG anti-βTG (range, 53–3300 kilounits/L) were found in patients with SLE (increased anti-dsDNA) and in patients with SSA/SSB antibodies (but with anti-dsDNA within reference values; Fig. 1). It is remarkable that these are the very nuclear antigens present in the blebs in the surface of apoptotic cells, which are the precursors of apoptotic bodies (16). The IgA anti-βTG concentration was 5–16 kilounits/L in the SLE sera and 18–58 kilounits/L in the SSA/SSB group. From these results one can conclude that celiac disease in unlikely, at least in the majority of the patients.

The apoptotic bodies and several components of their contents are autoimmunogenic (17). Tissue βTG is also present in the apoptotic bodies (1, 4), but that does not necessarily explain why it also is immunogenic.

Immunoglobulins against βTG substrate proteins have been demonstrated in various autoimmune diseases. One example is the presence of antibodies against gliadin, the protein that causes the symptoms in celiac disease, which is a preferred βTG substrate (18). It is supposed that dietary gliadin in gliadin-βTG complexes creates antigenic neoepitopes, which can initiate an immune response directed to both gliadin and βTG (19). Autoantibodies often are directed against different components of the same molecular complex (5, 8). A similar mechanism may cause the simultaneous formation of antibodies against βTG and its substrates (of which little is known) during apoptosis. βTG-substrate complexes typically exist only intracellularly, thus avoiding contact with the immune system. However, in situations of an imbalance between the supply and clearance of apoptotic bodies, the immune system can detect them, leading to an autoimmune response. This mechanism was demonstrated in SLE, where nucleosomes, which usually are cleared rapidly, expose the elementary antigens, leading to the formation of anti-DNA antibodies (12).

Our findings seem to fit into the theory that in certain autoimmune diseases, such as SLE, the content of apoptotic bodies comes into contact with the immune system, leading to an autoimmune response.

The difference in IgG anti-βTG concentrations between the SLE or SSA/SSB groups and the other groups is striking; patients with other detectable anti-nuclear antibodies and anti-proteinase 3 antibodies had normal or only slightly increased IgG anti-βTG concentrations (range, 0–55 kilounits/L). Perhaps the pathogenic mechanism in the latter groups of autoimmune diseases is different.

In the SLE patients, we did not find a longitudinal correlation between the concentrations of anti-DNA antibodies, which are useful in the follow-up of these patients, and anti-βTG. This suggests that anti-βTG may provide additional clinical information. In patients with Sjögrens...
syndrome, the concentrations of SSA/SSB antibodies do not correlate with exacerbations (20). Perhaps IgG anti-tTG has clinical value in monitoring these individuals.

Our results are only the first step in exploring the clinical value of IgG anti-tTG assays in patients with autoimmune diseases. In addition to the more fundamental aspects concerning the link between apoptosis and autoimmunity, its role in diagnosis, including sensitivity and specificity, and in the monitoring of patients still has to be elucidated and is the object of further investigations.

References


S100B Protein Concentrations in Amniotic Fluid Correlate with Gestational Age and with Cerebral Ultrasound Scanning Results in Healthy Fetuses, Diego Gazzolo,1 Matteo Bruschettini,1 Valentina Corvino,2 Renzo Oliva,3 Rossana Sarli,3 Mario Lituania,4 Pierlugi Bruschettini,1 and Fabrizio Michetti1 (Departments of 1 Pediatrics and 4 Obstetrics and Gynecology, Gianna Gaslini Children’s University Hospital, I-16147 Genoa, Italy; 2 Institute of Anatomy, Catholic University, I-00168 Rome, Italy; 3 Department of Obstetrics and Gynecology, Genoa University Hospital, I-16121 Genoa, Italy; 5 Laboratory of Immunohematology, Liguria, I-16142 Genoa, Italy; * address correspondence to this author at: Institute of Anatomy, Catholic University, Largo Francesco Vito 1, I-00168 Rome, Italy; fax 39-0630154813, e-mail fabrizio.michetti@rm.unicatt.it)

S100B is an acidic calcium-binding protein of the EF-hand family present in the central nervous system, where it is concentrated mainly in glial cells (1). It has been suggested that this protein is involved in various cellular functions (e.g., cell-cell communication, cell growth, cell structure, energy metabolism, and intracellular signal transduction) and that it may also act as a cytokine with neurotrophic effects at physiological concentrations. In this regard, studies in experimental models on laboratory animals and cell cultures have shown that decreased S100B expression in the nervous tissue correlates with neurobehavioral abnormalities and with microcephaly as a result of in utero cocaine exposure (2, 3). In humans, umbilical blood cord concentrations of S100B have been shown to be inversely correlated with gestational age, suggesting a neurotrophic role for this protein in the third trimester of pregnancy (4). On the other hand, its appearance at high concentrations in biological fluids has been shown to be a reliable marker of brain lesion in adults and pediatric patients and, recently, in the perinatal period (5–8). In particular, the appearance of S100B protein in the amniotic fluid of anencephalic fetuses is considered an indicator of damage in the central nervous system associated with neural tube defects (9).

This study provides reference values of S100B amniotic fluid concentrations during the second trimester of pregnancy.

We investigated, between the 15th and 18th weeks of gestation (mean, 16.5 weeks), 322 women (mean age, 35.5 ± 2.7 years; <35 years, n = 121; >35 years, n = 199) with consecutive physiological singleton pregnancies, who underwent amniocentesis for chromosomal abnormality exclusion (from June 1995 to November 1997). Appropriate fetal growth was defined by the presence of ultrasonographic signs (when the biparietal diameter and abdominal circumference were between the 10th and 90th centiles) according to the nomograms of Campbell and Thoms (10) and by postnatal confirmation of a birth weight between the 10th and 90th centiles according to our population standards after correction for the mother’s height, weight, and parity and the sex of the newborn. Exclusion criteria were multiple pregnancies; intraterine