Antibody formation against human collagen and C1q in response to a bovine collagen implant

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Summary. The humoral immune response to commercially available bovine collagen implants (Zyderm, Zyplast) is characterized in a 45-year-old female patient. Circulating anti-collagen antibodies were detected after eight injections of Zyderm and after two injections of Zyplast given during a period of 3 years. The specificity of these antibodies for bovine and human collagens as well as for the collagen-like region of C1q (a subcomponent of the first component of complement), was investigated by affinity chromatography. Serum levels of anti-collagen and anti-C1q antibodies were measured using ELISA. High levels of antibodies to bovine collagens, showing a strong cross-reactivity with human collagen type III were detected in the patient’s serum. Only weak cross-reactivity with human collagen type I and IV and no reactivity with type II were observed. In addition, these antibodies specifically cross-reacted with the collagen-like region of C1q. The antibody levels decreased continuously and disappeared 1 year after cessation of treatment. These results demonstrate for the first time the formation of autoantibodies upon treatment with a bovine collagen implant. Although antibodies to collagens and C1q have been found in various autoimmune diseases, neither adverse reactions to the bovine collagen implant nor any other clinical symptoms were observed in association with the described antibody response.

Key words: Bovine collagen implant — Immune response — Autoantibodies — Human collagen — C1q

Bovine collagen implants (BCI) are widely used for soft tissue augmentation in the treatment of acne scars, wrinkling and other skin defects. This treatment modality consists of intradermal injections of highly purified pepsin-solubilized bovine collagen preparations composed of more than 95% type I and less than 5% type III collagen.

Materials and methods

Patient

A 45-year-old female patient had received eight injections of Zd and two injections with Zp for cosmetic reasons, given mainly in the perioral region by different dermatologists since 1986 over a 3-year period.
Antibodies to native human collagens types I, II, III and IV and to pepsin-extracted calf skin collagen were determined in serum by a sensitive ELISA method as described previously [13]. Antibodies directed against the collagen-like region of Clq (CLR) were detected by an analogous technique using pepsin-digested Clq as antigen [15]. Results are expressed as the number of standard deviations of a normal control population above the normal mean, as described by Wener et al. [19]. Circulating immune complexes were measured by an ELISA version of the Clq solid phase RIA for immune complexes [8]. Aggregated IgG was used as a standard. RF's were determined by latex agglutination. All assays were performed in duplicate.

Affinity chromatography

Adsorption of sera to Zd and Zp. Serum (0.5 ml) diluted 1:20 with PBS was added to a miniature BCI affinity column consisting of 5 mg Zd or Zp retained in a Pasteur pipette by a tiny cotton-wool plug. The serum samples were passed through the columns at room temperature over a period of 30 min. Unbound protein was washed through the column with PBS containing 0.02% sodium azide.

Isolation of antibodies to human collagen type III. To isolate antibodies cross-reacting with native human type III collagen, an affinity column containing the antigen coupled to BrCN-activated Sepharose (LKB, Pharmacia, Uppsala, Sweden) was used. Collagen type III (8 mg) from human placenta (Sigma, St. Louis, USA) was coupled to 1 g Sepharose according to the manufacturer’s instructions. Coupling efficiency was 94%. The gel volume was 3.5 ml and 2.5 ml of serum sample was passed through the column. Bound antibodies were eluted first at pH 4.0 (0.1 M acetic acid, 0.15 M NaCl) and then at pH 2.8 (0.05 M glycine-HCl, 0.1 M NaCl). Elution of proteins was monitored photometrically at 280 nm. Acid-eluted antibody fractions were immediately neutralized and protected by addition of 0.5% bovine serum albumine (BSA). ELISA was immediately performed at the same final dilution (1:20) as used in the analysis of native sera.

Results

The patient had received eight injections with Zd over a period of 3 years, followed by two final treatments with Zp (Fig. 1). According to information from the patient, skin testing was performed prior to the first injection of Zd and no pathological reaction was observed. During the time of therapy no side-effects were observed. The first serum sample, S1, was obtained 5 days before the last BCI injection, and six additional blood samples were drawn during a follow-up period of 1 year.

At the beginning of the investigation the serum level of antibodies to bovine collagen was 12.3 SD above the normal mean. Subsequently, a continuous reduction was observed until the level was below the normal range (defined as 2 × SD above the normal mean) at the end of the observation period (Table 1). A similar time course was demonstrated for antibodies to CLR (17.5 × SD at the beginning, and 1.1 × SD at the end of the investigation; Table 1). Elevated levels of circulating immune complexes were detected only at the first three test points. All serum samples lacked RF activity (Table 1).

Figure 2 shows the time course of antibodies to human collagen types I–IV detected by ELISA. At the beginning of the investigation high levels of antibodies to human collagen type III were detected (22 × SD above the normal mean), whereas antibodies against types I and IV hardly exceeded the normal range. No elevated serum levels of antibodies against collagen type II could be detected. In a similar manner to the time course of antibody levels to bovine collagen and CLR, antibodies to human collagen type III decreased rapidly in the first 2 months and reached normal levels at the end of the observation period (Fig. 1).

Antibodies cross-reacting with human collagen type III were isolated from serum samples S1 and S2 by affinity chromatography. The first sample (S1) contained medium-affinity as well as high-affinity antibodies to human collagen type III, eluting at pH 4.0 and 2.8, respectively. Furthermore, at pH 4.0, antibodies to CLR were eluted from the column. In the second serum sample (S2) anti-type III antibodies could be desorbed at pH 4.0 but not at pH 2.8, indicating that cross-reactivity with human collagen type III was rapidly deteriorating after cessation of the BCI treatment. No significant amounts of anti-