SERUM AMYLOID P COMPONENT BINDS TO CELL NUCLEI IN VITRO AND TO IN VIVO DEPOSITS OF EXTRACELLULAR CHROMATIN IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Serum amyloid P component (SAP) is a normal plasma glycoprotein that is identical to, and the precursor of, amyloid P component (AP) found in deposits of all forms of amyloid in which it has been sought (1, 2). SAP and C-reactive protein (CRP), the classical acute-phase reactant, are very similar in molecular configuration, ultrastructural appearance, and amino acid sequence, and together form a distinct family of proteins called pentraxins (1, 3). Proteins homologous with SAP and/or CRP are present in all vertebrates in which they have been sought and neither polymorphism of SAP nor a deficiency state of SAP has been described in man. These features suggest that SAP has an important physiological function, although this is not yet known.

SAP exhibits specific calcium-dependent binding to a variety of ligands in vitro, including the pyruvate acetal of galactose, isolated amyloid fibrils, keratin filament aggregates, C4 binding protein, fibronectin, and some glycosaminoglycans (4–8). In vivo, a protein that crossreacts immunochemically with SAP is a normal matrix glycoprotein of glomerular basement membrane (9) and is also invariably found in noncovalent association with elastic fiber microfibrils in normal adult human tissues (10, 11). In view of the binding of SAP to fibronectin and glycosaminoglycans (7, 8), this normal tissue form of amyloid P component (TAP) may be involved in the maintenance of connective tissue architecture (11). More recently, it has been found that SAP is the single major serum protein that undergoes specific calcium-dependent binding to DNA and chromatin in vitro (12). In contrast, and contrary to a previous report (13), CRP was not found to bind significantly to either DNA or chromatin under physiological conditions of ionic strength (12). In the light of these observa-

tions, it was suggested that SAP may have a role in the handling of chromatin released from damaged and dying cells in vivo (12). We now report that SAP does indeed bind to extracellular chromatin in vivo.

Materials and Methods

Subjects and Skin Specimens.   Normal human serum was obtained from volunteers. Normal human skin specimens were obtained at surgical operations, and lesional skin from patients with discoid (DLE) and systemic (SLE) lupus erythematosus was obtained at the time of diagnostic biopsy.

Antisera and Chemical Reagents.   FITC-sheep anti-human SAP antibody was prepared and used as previously described (9). Other antibodies were obtained as follows: FITC- and/or rhodamine-conjugated (RITC)-rabbit anti-human IgG, IgM, IgA, and C3 (Dakopatts, Copenhagen, Denmark); antikeratin mAbs AE-1/AE-3 (Hybritech Inc, San Diego, CA) and CK 1 (Dakopatts); FITC-sheep anti-mouse IgG/IgM (Grub, Scandic, Vienna, Austria). Ethidium bromide was purchased from ʼSigma Chemical Co. (St. Louis, MO), and diamidine-phenylindole-dihydrochloride (DAPI) from Boehringer Mannheim Biochemicals (Mannheim, West Germany).
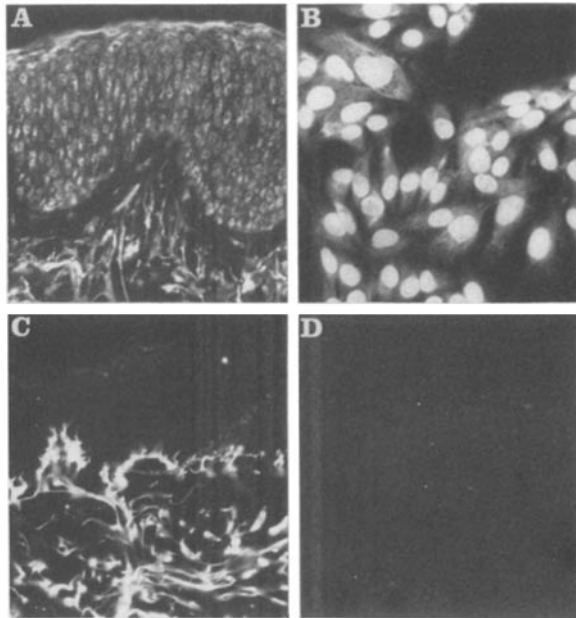
Immunofluorescence Staining of Normal Skin and Hep-2 Cells.   6-μm vertical cryostat sections of normal human skin, and monolayers of the Hep-2 epithelial cell line grown on glass slides (Kallestad Laboratories, Austin, TX) were incubated with normal human serum (either un-diluted or at 1:2) as a source of SAP for 30 min at room temperature. The substrates were then washed extensively, incubated with FITC-anti-SAP antibody for a further 30 min, followed by further extensive washing. The serum and antibody dilutions and all washes were carried out using either "calcium buffer" (0.01 M Tris, 0.138 M NaCl, 0.002 M $CaCl_2$, pH 8.0) or "EDTA buffer" (0.01 M Tris, 0.138 M NaCl, 0.01 M EDTA, pH 8.0).

Histochemical and Immunohistochemical Staining of Skin from Patients with Lupus Erythematosus. Cryostat sections from lesional skin of patients with DLE or SLE were stained using FITC-anti-SAP and/or by a double-immunofluorescence technique with a combination of FITC-anti-SAP and RITC-anti-IgM. Fluorescence histochemical stains used included 0.01% wt/vol ethidium bromide and 0.1 μg/ml DAPI stain to detect the presence of DNA. In two patients with SLE, in whom there were deposits of globular extracellular material in the upper dermis, sections were stained with: alcian blue, periodic acid Schiff stain, Congo Red, orcein, Giemsa, toluidine blue, Feulgen stain, and thioflavine T (fluorescence). Immunoperoxidase staining with CK1 antikeratin antibody, and immunofluorescence staining with FITC-anti-human IgG, IgM, IgA, and C3, and AE1/AE3 antikeratin antibodies followed by FITC-sheep anti-mouse IgG/IgM were also carried out.

Results

Immunofluorescence Staining of Normal Skin and Hep-2 Cells.   Preincubation of cryostat sections of normal human skin, and cultured Hep-2 cells, with normal human serum (as a source of SAP), followed by staining with FITC-anti-SAP, resulted in nuclear fluorescence of epidermal keratinocytes and of the Hep-2 cells. This was only observed in the presence of calcium (Fig. 1, *A* and *B*) and not with EDTA buffer (Fig. 1, *C* and *D*). Calcium-dependent cytoplasmic fluorescence of keratinocytes and of Hep-2 cells, on preincubation with normal human serum followed by staining with FITC-anti-SAP, probably resulted from SAP binding to keratin intermediate filaments, as previously reported (6). The expected calcium-independent fluorescence of dermal elastic tissue with FITC-anti-SAP (10) was also observed in normal human skin.

Histochemical and Immunohistochemical Staining of Skin from Patients with Lupus Erythematosus.   Neither DNA nor SAP was demonstrable in the lupus band in any of the biopsies studied. However, in one patient with SLE hematoxylin and eosin staining of a biopsy from an erythematous elbow papule showed remarkable globular deposi-

FIGURE 1. Immunofluorescence staining with FITC anti-SAP antibody of normal human skin and Hep-2 cells, preincubated with normal human serum as a source of SAP. Note nuclear and cytoplasmic staining of keratinocytes (A) and staining of nuclei and cytoplasmic filaments of Hep-2 cells (B) in the presence of calcium; this staining is absent in keratinocytes (C) and Hep-2 cells (D) when EDTA buffer is used. Fluorescence of dermal elastic tissue is seen under both conditions (A, C). A, C ×250; B, D ×500.

tion of basophilic material in the upper dermis associated with a marked neutrophil polymorphonuclear cell infiltrate and leukocytoclasis (Figs. 2 and 3 A). Biopsy of a papular purpuric eruption on the leg of a second SLE patient also showed globular deposition of basophilic material in the upper dermis, associated with an extensive leukocytoclastic vasculitis and dermal infarction (not shown). In both biopsies these unique dermal deposits stained for DNA with ethidium bromide (Fig. 4 A), and/or DAPI (Fig. 3 B) or Feulgen stain. These deposits also showed bright, specific fluorescence with FITC–anti-SAP antibodies, indicating co-deposition of chromatin and
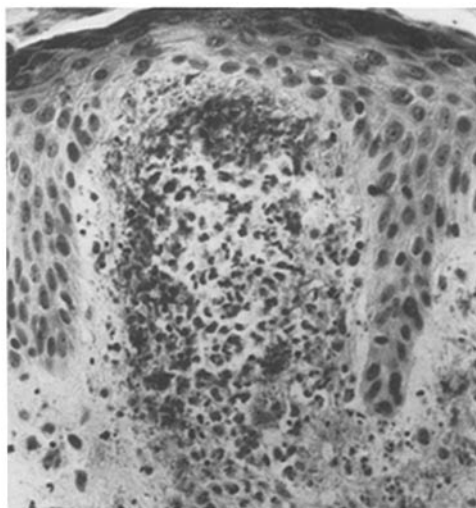
FIGURE 2. Biopsy of lesional skin from SLE patient A; hematoxylin and eosin stain. Survey micrograph showing extensive neutrophil polymorph infiltrate and leukocytoclasis associated with globular material in the upper dermis. ×320.
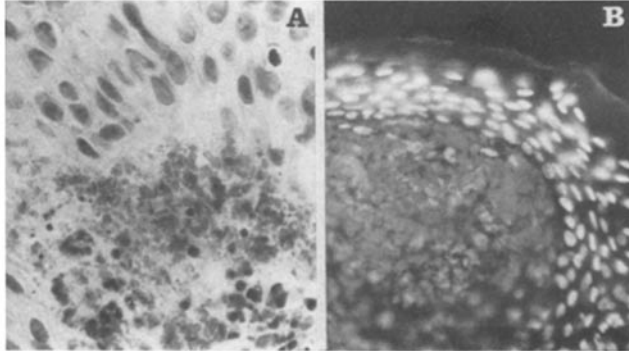
FIGURE 3. Lesional skin from SLE patient A. (A) High power view showing basophilic globular material in the upper dermis; hematoxylin and eosin stain. (B) DAPI staining. Note bright fluorescence associated with keratinocyte nuclei, and weaker fluorescence associated with DNA material within the dermal globular deposits. A, ×800; B, ×320.
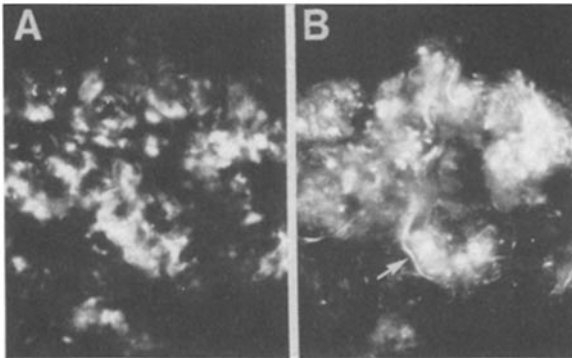

FIGURE 4. Double-fluorescence staining of area of dermis from lesional skin of SLE patient B with (A) ethidium bromide and (B) FITC anti-SAP antibody. Note overlap in staining pattern with both fluorochromes, although elastic fiber staining is seen only with anti-SAP (arrows). A and B, ×300.
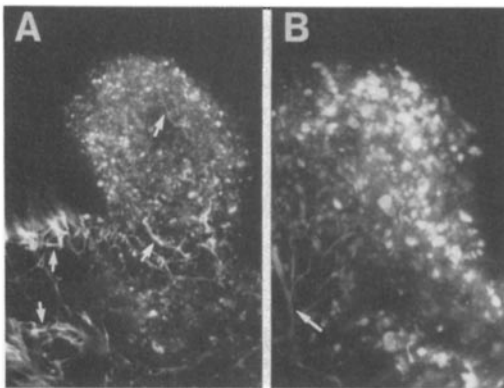

FIGURE 5. Immunofluorescence staining of lesional skin from SLE patient A with FITC anti-SAP antibody. Note bright fluorescence of the dermal globular material, in addition to the expected fluorescence associated with fibers of the elastic tissue system (arrows). A, ×250, B, ×500.

SAP in vivo (Figs. 4 B and 5). Bright staining with anti-IgM and anti-IgA, and faint with anti-IgG and anti-C3, was also seen (not shown); this may have reflected the presence of antinuclear autoantibodies. None of the other histochemical stains used gave positive results.

Discussion

It has recently been reported that SAP is the only plasma protein that, from the

milieu of whole human serum, undergoes specific calcium-dependent binding to chromatin and DNA in vitro (12). In the present study we have extended this observation by showing that SAP from serum undergoes calcium-dependent binding to nuclei of Hep-2 cells and of keratinocytes in sections of normal skin. As predicted from our recent finding of calcium-dependent binding of SAP to keratin filaments (6), SAP from serum also bound to cytoplasmic structures in keratinocytes and to cytoskeletal filaments of Hep-2 cells. Dermal elastic fibers in normal human skin were stained with anti-SAP antibody as previously described (10).

In a murine model of SLE, ultraviolet light B (UV-B) treatment of mice sensitized to UV-B-denatured DNA resulted in the deposition of immunoreactants along the basement membrane zone (14). It has therefore been suggested that the lupus band occurs in DLE and SLE because UV-denatured DNA from damaged epidermal cells is released into the upper dermis and reacts there with circulating anti-DNA autoantibodies (14, 15). In all seven of our patients with lupus erythematosus in whom there was a proven IgM^+ lupus band, there was, however, no staining in a pattern corresponding to the location of the lupus band with either stains for DNA (ethidium bromide or DAPI) or with FITC–anti-SAP antibody.

In contrast, two of these SLE patients had remarkably extensive deposits of DNA-containing nuclear material in the upper dermis (identified by ethidium bromide, DAPI stain or Feulgen stain) that also stained specifically and intensely with FITC–anti-SAP antibodies. The deposits did not stain with antikeratin antibodies, indicating that the presence of SAP was not due to the previously reported binding of SAP to keratin filaments (6). There is thus clear evidence for the deposition of SAP in association with extracellular chromatin in vivo, supporting the idea (12) that SAP may be involved in the disposal of this biologically important material. The potential of "free" chromatin for causing deleterious effects is considerable, both as a significant autoantigen and as a source of inappropriate genetic information if the DNA were to enter cells. Further experiments to define the possible role of SAP in modulating such effects are in progress.

Summary

Serum amyloid P component (SAP) is the single plasma protein that, from the milieu of whole normal human serum, undergoes specific calcium-dependent binding to isolated DNA and chromatin in vitro. We now report for the first time that SAP in whole serum also undergoes calcium-dependent binding to nuclei of epidermal cells in sections of normal human skin and to nuclei of fixed Hep-2 cells, a human epithelial cell line. Furthermore, and most importantly, SAP was detected in association with unusual globular dermal deposits of nuclear material in skin biopsies from two patients with systemic lupus erythematosus. This is the first evidence for binding of SAP to extracellular chromatin in vivo and supports the idea that SAP may have an important physiological role in the disposal of this material.

References

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