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New immunoassays for the selective measurement of serum amyloid A isoforms 1 and 2 in human blood

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Introduction

The SAA is an acute phase protein and a sensitive marker of inflammation and tissue damage. Under inflammatory conditions, the concentration of SAA in blood increases by up to 1,000-fold.

In humans, acute phase SAA is represented by two isoforms: SAA1 and SAA2. It has been reported that *SAA1* and *SAA2* expression levels may vary in response to different inflammation stimuli (Thorn et al, 2003) and it was also suggested that the differential measurement of SAA isoforms might have additional clinical value.

SAA1 and SAA2 share 92% identity in the primary structures. Existing immunochemical methods for SAA measurement detect both SAA isoforms. The development of immunochemical methods for the selective measurements of SAA1 and SAA2 concentrations has not yet been reported.

The aim of this study was to develop three sandwich immunoassays: an immunoassay specific for SAA1; an immunoassay specific for SAA2; and an immunoassay specific for the total SAA, which recognizes both SAA isoforms with the same level of efficiency.

Methods

Recombinant SAA1 (SAA1.1) and SAA2 (SAA2.1) that contained no additional amino acid residues except for N-terminal methionine were expressed in *E. coli* and purified by immunoaffinity chromatography under identical conditions. Mouse monoclonal antibodies (MAbs) SAA80, SAA100, 2SAA65, and VSA31, were obtained from HyTest.

MAbs conjugated with europium chelate were used as labels in sandwich immunoassays for the detection of SAA. Recombinant SAA1 was used as a calibrator for assays that were specific for SAA1 and the total SAA, while recombinant SAA2 was used as a calibrator for the SAA2 assay.

EDTA plasma samples were collected from 28 patients who underwent surgery within 7 - 62 hours of the surgery taking place.

Results

We determined the MAb epitopes by using a set of synthetic peptides (Fig. 1). The epitope of the MAb SAA80 was located in the region 27-50 aar; the epitope of the MAb VSA31 was located in the region 84-101 aar. Both of the MAbs SAA80 and VSA31 equally recognized SAA1 and SAA2.

The epitopes of the MAbs SAA100 and 2SAA65 were located in the region 73-88 aar. The MAb SAA100 was specific to SAA1, while the MAb 2SAA65 was specific to SAA2. No cross-reactivity with the other isoform was observed for either of these MAbs. Region 73-88 aar contains residue 84, which differs between SAA1 and SAA2 (Glu/Lys). We speculated that Glu84 and Lys84 are critical for the binding of the MAbs SAA100 and 2SAA65, respectively.

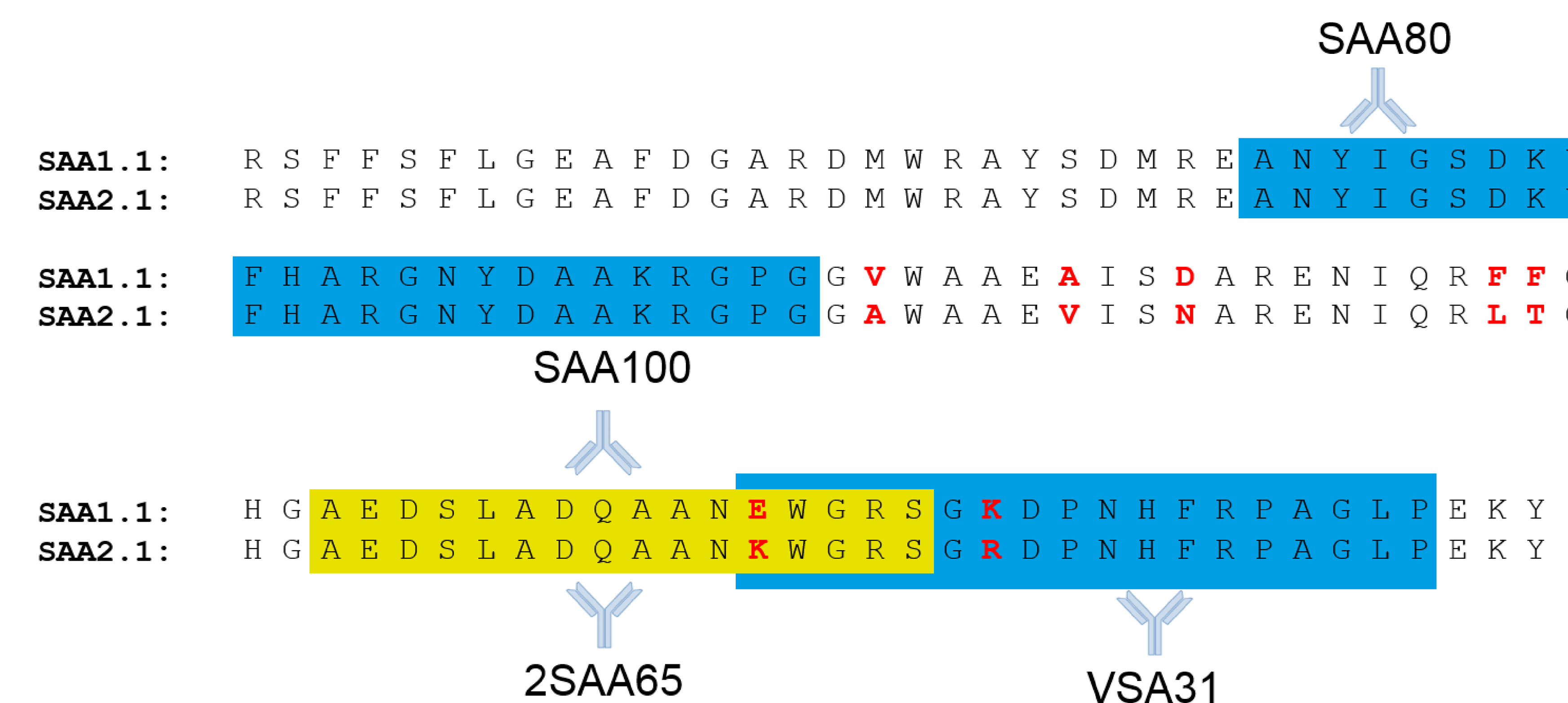

FIGURE 1. The primary structure of acute phase isoforms SAA1 and SAA2. Variable aar are shown in a red font. The frames show the location of the MAb epitopes.

The monoclonal antibody SAA80, which equally recognizes both SAA isoforms, was used as a capture antibody in all of the assays. Meanwhile, the MAbs SAA100, 2SAA65, and VSA31 were used as the detection antibodies (Fig. 2).

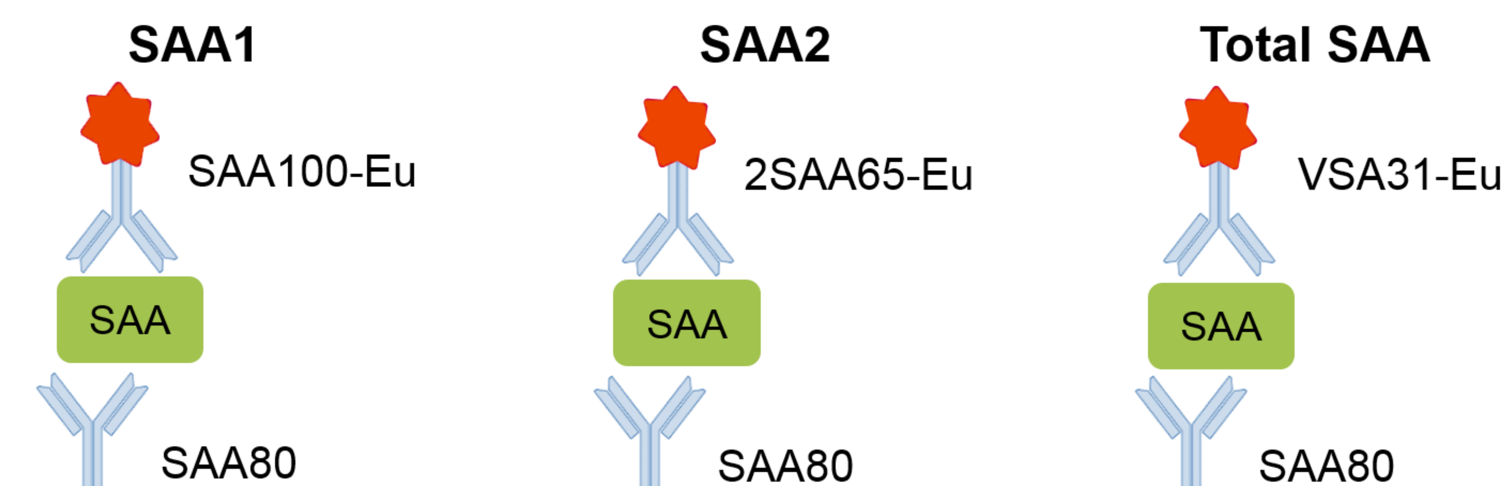

FIGURE 2. Schematic representation of immunoassays used for the measurement of SAA1, SAA2, and the total SAA.

The immunoassay SAA80-SAA100 for the measurement of SAA1 showed no cross-reactivity with recombinant SAA2 (Fig. 3), whereas the immunoassay SAA80-2SAA65 for the measurement of SAA2 showed no cross-reactivity with recombinant SAA1. The immunoassay SAA80-VSA31 for the measurement of the total SAA level detected both isoforms with almost the same level of efficiency (cross-reactivity with SAA2 was 93%). The detection range of the immunoassays was similar, and it corresponded to 1-50 ng/ml.

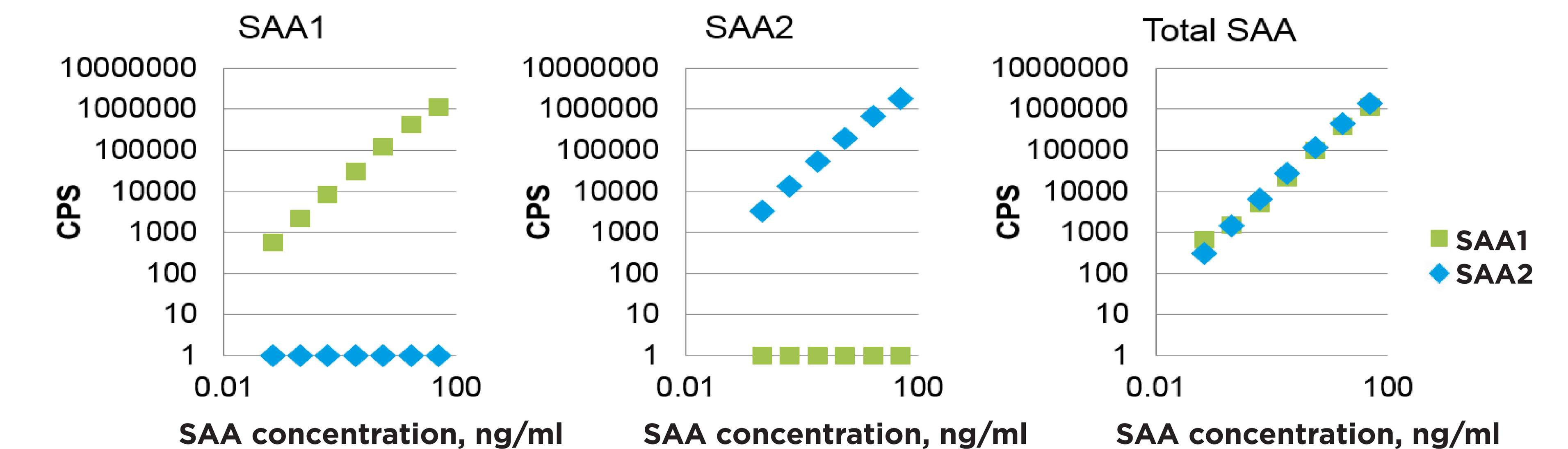

FIGURE 3. Cross-reactivity of developed immunoassays with SAA1 and SAA2.

Developed assays were used in order to evaluate the content of SAA isoforms in the plasma of patients with inflammation caused by tissue injury. The total SAA concentration in plasma samples ranged from 2.6 to 293.0 mg/L (median 12.8 mg/L). SAA1 was the predominant isoform in all of the tested samples (Fig. 4). The SAA1/total SAA ratio was 80.4±5.5% (mean±SD), while the SAA2/total SAA ratio was 15.9±7.1% (mean±SD). The sum of SAA1 and SAA2 concentrations that were determined by isoform-specific assays comprised 96.3±5.8% (mean±SD) of the total SAA concentration for each patient. This confirms the reliability of the proposed method of detection.

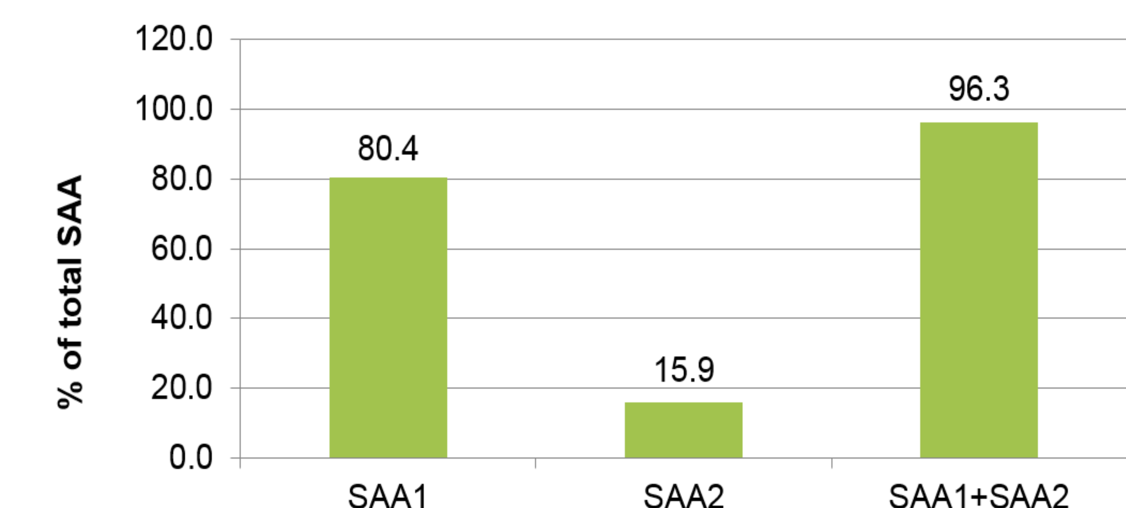

FIGURE 4. Relative content of SAA1 and SAA2 in the plasma of patients with inflammation. Results are provided as ratios of SAA1, SAA2, and the sum of selectively measured SAA1 and SAA2 concentrations to the total SAA concentration (mean±SD).

Conclusions

We have developed immunoassays for the selective measurement of SAA isoforms 1 and 2 that have not been described before. These immunoassays could be used for the quantification of SAA1 and SAA2 in patients with inflammation caused by different inflammatory stimuli in order to define the clinical value of the SAA isoforms.

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