Comparative study of C reactive protein and serum amyloid A protein in experimental inflammation

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Abstract

The responses of C reactive protein, measured by radial immunodiffusion and radioimmunoassay, and serum amyloid A protein, measured by radial immunodiffusion, were compared in eight subjects with inflammation induced experimentally by intradermal injection of monosodium urate crystals. A significant increase in serum amyloid A was noted after a lag phase of eight hours, the increase in median concentration at 48 hours being about eightfold. A parallel but less marked increase was found in C reactive protein when measured by radioimmunoassay (fourfold increase in median concentration at 48 hours) after a small but significant decrease during the lag phase. The changes in C reactive protein remained within the reference range and were not detectable by radial immunodiffusion.

Acute phase proteins are measured routinely in many clinical laboratories to assist in establishing the presence of inflammation or infection, and in assessing the activity of disease and its response to treatment. Many plasma proteins increase in concentration in response to inflammatory stimuli, but C reactive protein and serum amyloid A protein are probably the most suitable markers.¹ Not only do they undergo a rapid and substantial increase in concentration but they also have short half lives, thus decreasing rapidly when the stimulus ceases.¹ C reactive protein, in particular, is now measured extensively in clinical practice.² Serum amyloid A protein is less popular owing to lack of commercial antisera, calibrants, and suitable well characterised routine assays.³

Previous studies⁴⁻¹⁰ have indicated that serum amyloid A may be more sensitive than C reactive protein, sometimes rising above the reference range when C reactive protein apparently remained within it (<10 mg/l). In all these studies C reactive protein was assayed by techniques such as radial immunodiffusion, which have a lower limit of sensitivity of 5-10 mg/l. It is now clear from studies using sensitive radioimmunoassays that the C reactive protein concentration in most normal subjects is much lower—for example, median 0.58 mg/l, range 0.068-8.20 mg/l¹¹; median 0.80 mg/l, range 0.070-29.0 mg/l¹²; and, furthermore, the reference range is markedly skewed towards the lower limit. It is thus possible for C reactive protein to increase 10-100-fold but still remain undetectable by many routinely used techniques. This may account for its apparently lower sensitivity. Serum amyloid A protein is detect-

able by radial immunodiffusion in 99% of normal subjects,¹³ and increases are less likely to be masked by method insensitivity.

In a previous study of experimental inflammation we showed in certain subjects a response in serum amyloid A but not in C reactive protein when measured by radial immunodiffusion.¹⁴ We have therefore re-examined the C reactive protein response with a sensitive radioimmunoassay to determine whether changes could be detected within the reference range.

Patients and methods

The induction of inflammation by intradermal injection of monosodium urate crystals has been described in full previously.¹⁴ Eight subjects were included in this study. Serum was separated from clotted blood taken at 0, 4, 8, 24, 32, and 48 hours and stored at -70°C. C reactive protein was measured by conventional radial immunodiffusion¹⁵ and by a double antibody radioimmunoassay using an iodinated label.¹¹ The correlation between the two C reactive protein assays at concentrations >10 mg/l was $r=0.99$ (170 paired determinations). Serum amyloid A was measured by a modified radial immunodiffusion.¹³ The significance of the differences in protein concentrations before and after stimulation was determined by the Mann-Whitney U test.

Results

The figure shows the responses of C reactive protein and serum amyloid A. For serum amyloid A there were no significant changes ($p>0.05$) at four and eight hours, but concentrations at 24, 32, and 48 hours were significantly greater ($p<0.001$) than before stimulation. The median increase at 48 hours was about eightfold.

For C reactive protein (radioimmunoassay) no significant change in concentration was seen at four hours, but at eight hours the concentration was significantly lower ($p=0.02$) than before stimulation. The concentrations at 24, 32, and 48 hours were significantly greater ($p<0.001$) than before stimulation, the median increase at 48 hours being about fourfold.

C reactive protein measured by radial immunodiffusion was undetectable (<5 mg/l) at all times.

Discussion

This study clearly shows that significant increases in the serum concentration of C

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Median concentrations of serum amyloid A protein (●) and C reactive protein (measured by radial immunodiffusion (▲); radioimmunoassay (○)), in eight subjects after intradermal injection of monosodium urate crystals. Ranges are given for serum amyloid A and C reactive protein (radioimmunoassay). All radial immunodiffusion values for C reactive protein were <5 mg/l. SAA=serum amyloid A; CRP=C reactive protein; RIA=radioimmunoassay; RID=radial immunodiffusion.

reactive protein can be seen in response to a mild inflammatory stimulus, but the changes occur within the reference range (<10 mg/l) and are undetectable by conventional methods such as radial immunodiffusion. A more sensitive technique such as radioimmunoassay is required.

The response of C reactive protein parallels that of serum amyloid A, but is less marked (fourfold median increase at 48 hours C reactive protein, eightfold serum amyloid A). Thus serum amyloid A probably is the more sensitive acute phase protein with a greater incremental increase, a finding which confirms our previous observations in inflammatory bowel disease.⁶ If sensitivity is defined as the percentage of subjects in whom a response is demonstrable (positive result), that of C reactive protein (100%) is the same as that of serum amyloid A (100%). The apparently lower sensitivity of C reactive protein noted in previous studies⁴⁻¹⁰ may therefore be artefactual, arising from the use of insensitive assays (radial immunodiffusion) and an inappropriately high cut off point.

The well reported lag phase in the increase of C reactive protein¹⁶⁻¹⁷ is clearly visible by radioimmunoassay (figure). Also apparent is an initial decrease in concentration ($p=0.02$ at eight hours). Similar decreases during inflammation have been seen with other plasma proteins¹⁷ and are thought to be the result of increased microvascular permeability. None of these changes is visible by radial immunodiffusion. The lag phase of serum amyloid A is similar to that of C reactive protein,¹⁴ but, unlike C reactive protein, no initial decrease in

concentration was seen. This might have been owing to the insensitivity of the radial immunodiffusion assay for serum amyloid A in detecting changes at concentrations <10 mg/l, or to the high molecular weight of the serum amyloid A-high density lipoprotein complex in which most of the serum amyloid A circulates.

For many clinical circumstances in which C reactive protein estimation is of value the ability to detect changes occurring within the reference range is not critical as concentrations in the range 20–300 mg/l are often found.¹⁻² Methods such as radial immunodiffusion, or the more recently introduced immunoturbidimetric/nephelometric assays, are therefore entirely adequate.

There are other circumstances, however—for example, detecting intercurrent infection in neonates¹⁸⁻²⁰ or in immunosuppressed patients,²¹⁻²⁵ in which a more sensitive method would be advantageous. The inflammatory response would become apparent earlier and treatment could be started more promptly. Sensitive assays are also required for experimental investigations into the mechanisms of the inflammatory response.¹⁴⁻¹⁷ Serum amyloid A, being more sensitive than C reactive protein, would probably be the most suitable inflammatory marker, but its routine assay is impractical at present owing to lack of suitable antisera and calibrants. The results of our study presented here indicate that C reactive protein, when assayed by a sensitive method, would undoubtedly be a suitable alternative.

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