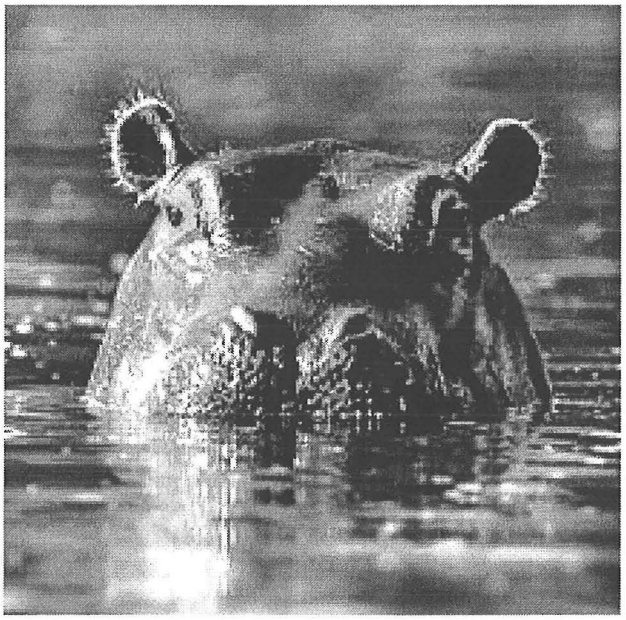
# C-Reactive Protein and Cardiovascular Risk:



## The Eyes of the Hippopotamus

Internal Medicine Grand Rounds

Parkland Memorial Hospital

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Robert S. Munford, M.D. has no financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Munford will be discussing "off-label" uses in his presentation.

Dr. Munford is Professor of Internal Medicine and Microbiology and holder of the Jan and Henri Bromberg Chair in Internal Medicine. His interests include bacterial endotoxin, host defenses vs. bacterial infection, sepsis, and physiologically-responsive gene therapy for inflammatory diseases.

Thanks to Dr. J. Tsai for her excellent resident's conference on this subject earlier this year (1).

Tillett and Francis discovered C-reactive protein (CRP) at the Rockefeller Institute in 1930. Students of pneumococcal disease, they found that a protein in serum bound to the pneumococcal "C" polysaccharide. Avery and others subsequently found that serum concentrations of the protein rose dramatically during acute illness. CRP was purified and crystallized by McCarty (1954) and its amino acid sequence was determined by Gotschlich and Liu (1978).

Following an acute stimulus, serum concentrations of CRP increase rapidly, peaking 24 – 72 hours later. The plasma half-life of CRP (19 hrs) is independent of the plasma concentration and seems to be constant in all conditions (2). So the major determinant of the plasma concentration is the rate at which CRP is produced and released into the circulation.

CRP has been known to clinicians for decades as a sensitive indicator of illness. It has competed with the erythrocyte sedimentation rate for purposes such as distinguishing "sick" from "not sick," discriminating bacterial from viral infections, gauging response to therapy for osteomyelitis, etc. A rejuvenation of interest in CRP was triggered by several recent studies that point to a role for this protein in cardiovascular disease.

My original goal for this Grand Rounds was to evaluate the recently published data on the use of C-reactive protein (CRP) to predict risk of cardiovascular disease. As I ventured deeper and deeper into the literature, however, a broader scenario came into view. CRP is an easily measured marker for tthe Acute Phase Response (APR), a highly conserved reaction to injury, infection, and other stresses. Remarkably, most of the known risk factors for atherosclerosis are also acute phase proteins. What could trigger the APR in seemingly healthy adults? Most of the evidence pointed to interleukin-6 as the dominant stimulus. So what elevates IL-6 levels in the blood? Numerous acquired influences (obesity, smoking, chronic infection, stress) all seem to trigger IL-6 release, and there are genetic factors as well. The outcome of my literature search is a web of causality, with IL-6 (+/- TNF-$\alpha$) at its center, that seems to provide plausible links between [local inflammation, injury, obesity, smoking, age] and [dyslipidemia, atherosclerosis, and metabolic syndrome X]. The possible implications of these links encompass much of modern internal medicine.

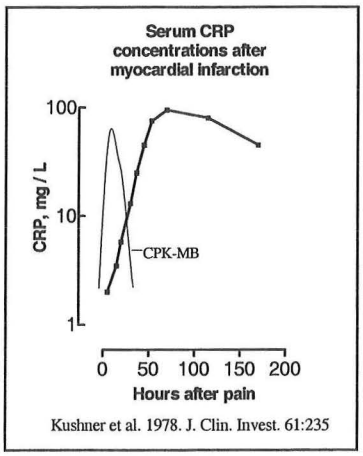
**CRP and the risk of atherosclerotic cardiovascular disease**

There have been 3 kinds of studies:

- Studies in patients with myocardial infarction. Risk of infarct extension, death.
- Studies in patients with angina. Short-term risk of MI, unrelenting angina, death.
- Population-based studies in healthy adults. Long-term risk of MI, stroke, peripheral vascular disease.

*Studies in patients with myocardial infarction* (3-8)

Myocardial infarction, like other kinds of tissue necrosis, provokes systemic responses that can include fever and leukocytosis in addition to acute phase protein synthesis. Serum CRP levels increase exponentially, with a doubling time of ~8.2 hours, then decay with a half-life of 19 hrs (2)(Figure). . Post-MI CRP levels can reach well over 100 mg/L. The time to peak CRP level is variable; intervals of 50 – 100 hours after infarction are typical. Given the apparent relationship between serum CRP concentration and the extent of myocardial injury (LDH (2), CK-MB (4,9), troponin I, hydroxybutyrate dehydrogenase) (7)), it's not surprising that very high CRP levels identify patients with lower ejection fraction (7,10) or post-MI complications (4). There is also interesting evidence that CRP may exacerbate myocardial damage by binding to infarcted myocardium and activating complement (11,12).



Kushner et al. 1978. J. Clin. Invest. 61:235

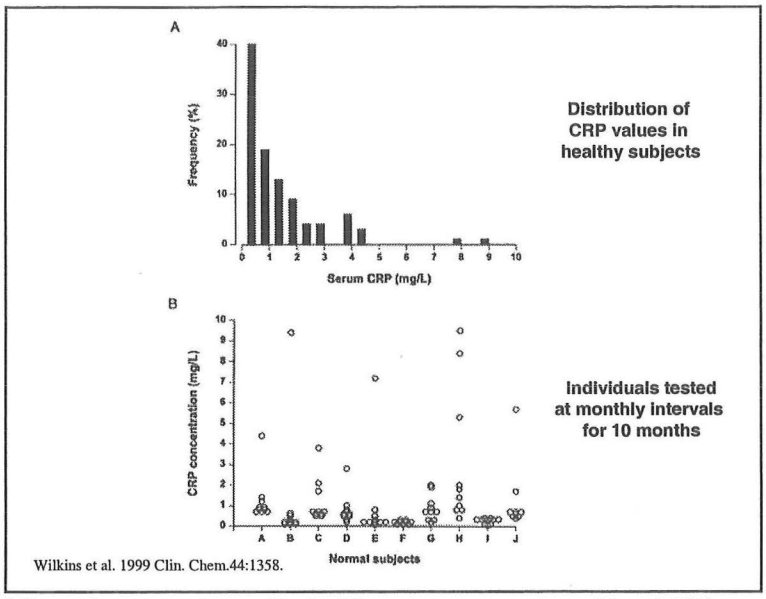
*Studies in patients with angina* (10,13-25)

The technological innovation that allowed these studies was the development of "high sensitivity" assays for CRP – assays that can measure CRP levels well below the previous detection limit, 10 mg/L. The assays are highly reproducible yet the CRP levels in a given individual can fluctuate over time (see Figure below, panel B). Most laboratories use an international (WHO) CRP standard.

It is also important to note that CRP levels in humans are not normally distributed (panel A at right). In many studies, the individual results are normalized (log) so that the values will be normally distributed (bell-shaped curve). Most population-based studies have divided the population into tertiles, quartiles, or quintiles – it

usually isn't obvious why a particular division was chosen. Using the Dade/Behring rate nepholometric method, the 50th percentile values for adult men and women blood donors are 0.7 and 0.9, respectively; the 75th percentile values are 1.4 and 3.1, respectively (26).

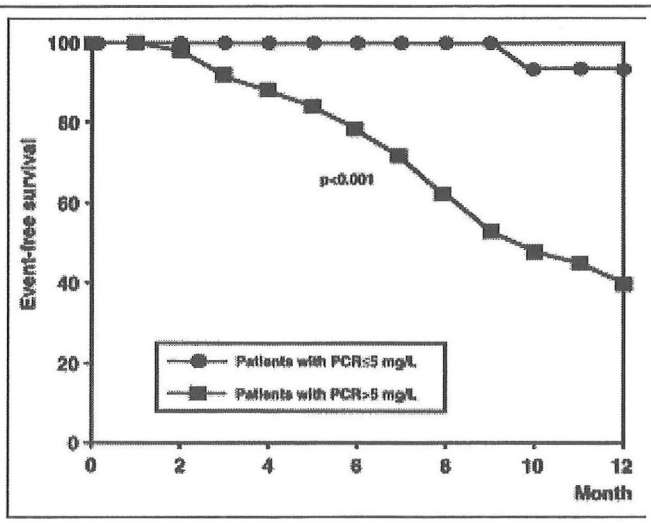
*In this Grand Rounds, CRP levels will be stated in milligrams/liter (mg/L), in keeping with the improvement in assay sensitivity.*



Wilkins et al. 1999 Clin. Chem.44:1358.

Several lines of evidence now suggest that inflammation within coronary arteries is an important component of unstable angina (27,28). When compared to patients with stable or atypical angina, those with unstable angina have increased expression of adhesion molecules on neutrophils and monocytes collected from coronary sinus blood (29) and greater tissue factor expression on circulating monocytes (30). Using a thermistor probe to measure the temperature of the vessel wall as close as possible to the offending intracoronary lesion, Stefanadis et al. found that the gradient [vessel wall temperature – oral temperature] was elevated in patients with unstable angina or myocardial infarction, and that this gradient also correlated well with blood CRP and SAA levels (31).

In patients admitted to intensive care units with unstable angina (and, by definition, no evidence for ongoing infarction), several groups have reported that admission CRP levels correlate positively with risk of subsequent cardiac events (MI, unrelenting angina requiring revascularization, death)(5,10,16-18,22). Other groups have not found such associations, however (20,21); the basis for this difference is not clear. In the studies that found an association between admission CRP and subsequent adverse events, the "cut-off" for high CRP level ranged from 3 to 10 mg/L. In a study of patients who had experienced a myocardial infarction within the preceding 3 – 20 months, a CRP level of >6.6 mg/L was associated with almost 2-fold increase in subsequent MI, relative to the risk associated with CRP <1.2 mg/L (32).

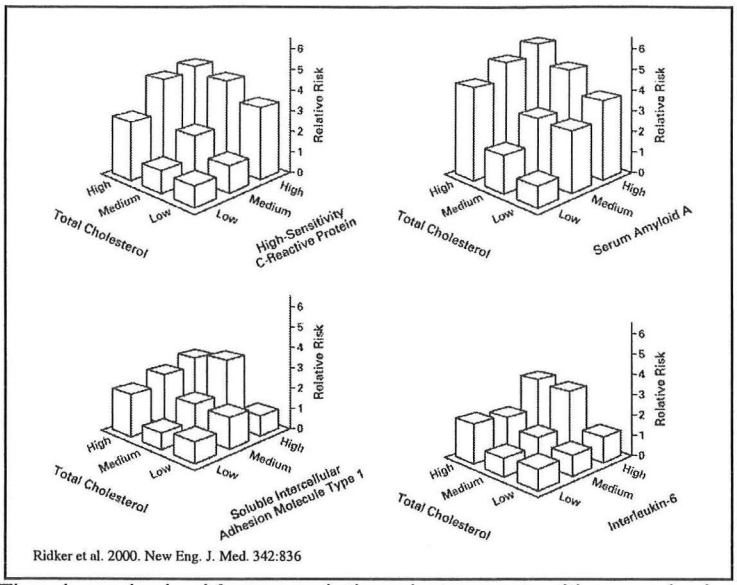


Event-free survival according to admission CRP in patients with UA who underwent coronary stenting.Versaci et al. 2000 Am.J.Cardiol. 85:92 ("PCR" = CRP)

Myocardial ischemia per se does not elevate CRP levels, in keeping with the notion that in patients with unstable angina the inflammation is in the vessel wall, not in the myocardium (19).

*Population-based studies in healthy adults* (33-43).

I reviewed 9 published studies. Almost all were retrospective analyses of data that had been collected for other reasons. In these studies, CRP was measured only at baseline; follow-up lasted as long as 17 years. A meta-analysis of prospective studies performed prior to 1998 concluded that "comparison of individuals with CRP values in the top third with those in the bottom third at baseline yielded a combined risk ratio for CHD of 1.7 (95% CI, 1.4 – 2.1)(44) . The estimated *mean* CRP values in these two groups are 2.4 and 1.0 mg/L, respectively. In most of the studies, CRP remained a strong predictor of subsequent cardiac events even when total cholesterol and HDL cholesterol were taken into account. All of the studies adjusted for smoking and lipids and some also adjusted for fibrinogen and other variables. The recently published analysis of CRP and other markers in predicting CAD in postmenopausal women (Figure below) suggests that CRP (or SAA) measurement may provide prognostic information in addition to that obtained from total cholesterol levels (40). The "low"

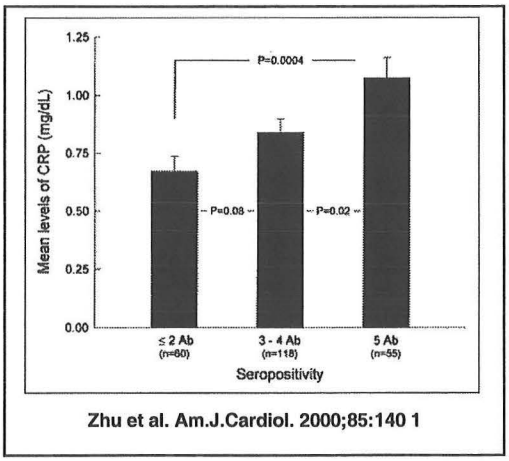


Ridker et al. 2000. New Eng. J. Med. 342:836

cholesterol group in this study had LDL cholesterol levels below 130 mg/L, and a mean LDL cholesterol of 104 mg/dl.

Although high-normal CRP levels have also been predictive of stroke (14,35,37,45)7060(46) they have not been consistently associated with high risk of peripheral vascular disease (14,47).

*Studies of associations between serum CRP levels and infection with specific microbial agents (40,48-53).* What triggers the the inflammation that elevates CRP levels in otherwise healthy individuals? Several groups of investigators have tried to establish a link between ongoing intravascular) infection and serum CRP levels. They have looked for associations between positive serologies (for *C. pneumoniae*, CMV, others), or blood endotoxin levels, and serum CRP. These studies have had inconsistent results; some have found statistically significant correlations with serum CRP levels, while others have not. Reports from two groups suggest that the "total burden" of infectious exposures correlates best with CRP levels (49,51). See Figure at right.



Zhu et al. Am.J.Cardiol. 2000;85:140 1

The other major possibility is that inflammation in non-vascular tissues is the stimulus that raises CRP levels. The lungs (especially in smokers), periodontal tissues, and adipose tissue (see below) are the leading candidates.

See Dr. John Rutherford's Grand Rounds (July 23, 1998) for an excellent discussion of these issues.

## Do drugs lower that prevent atherosclerosis also lower CRP?

1. *Aspirin and NSAIDS.* Aspirin has both anti-platelet and anti-inflammatory activities. In men participating in the Physicians' Health Study, taking aspirin (325 mg qod, Bufferin) was associated with a statistically significant reduction in relative risk of myocardial infarction. The reduction was insignificant in the lowest quartile of baseline CRP values, 33.4% in the second quartile, 46.3% in the third quartile, and 55.7% in the fourth quartile (35). Although this analysis has been criticized for lack of rigor (44), it suggests that at least part of the beneficial effect of aspirin may be due to its anti-inflammatory potency. Does aspirin lower CRP levels? When it was given to healthy men for 7 days, 81 or 325 mg aspirin did not lower pre-exercise or post-exercise CRP levels (54). Similarly, pre-treatment with ibuprofen or indomethacin did not diminish the CRP response to an intravenous endotoxin challenge in human volunteers (55). On the other hand, a 3-week course of 300 mg/day did reduce IL-6 and CRP levels in men with angina and exercise-induced ischemic EKG changes (56). Aspirin thus may decrease CRP levels when it is given for a few weeks; a study with longer follow-up is needed.

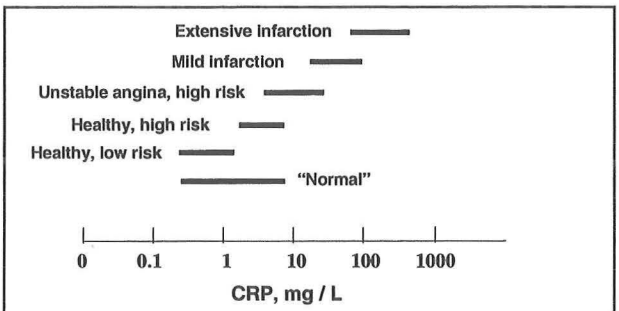
2. *Statins.* The CARE trial of secondary prevention randomized post-MI patients to receive 40 mg/day pravastatin or placebo. In a retrospective analysis of this trial, there was an association between high baseline levels of CRP (and SAA) and recurrent M.I. The proportion of recurrent coronary events prevented by pravastatin was 54% in the subgroup with inflammation (>90th percentile of both CRP and SAA) and 25% in the subgroup without inflammation (32). (It's not clear that this subgroup analysis was specified in the analytical plan for the study, however.) In another analysis of the CARE data, the same group found that median CRP levels tended to increase over 5 years in placebo recipients, whereas median CRP levels decreased by 17% in those allocated to pravastatin. At 5 years, the difference in median CRP levels (21.6%), mean CRP levels (37.8%) and the absolute mean change in CRP (0.137 mg/dl) between placebo

and pravastatin groups were highly significant (57). The differences persisted after stratification by baseline lipid levels and other standard risk factors, and there was no obvious relationship between the magnitude of change in CRP and the magnitude of change in lipids. A smaller study from Finland also described decreases in CRP associated with statin use (58).

HMG CoA-reductase inhibitors may have numerous putatively anti-atherogenic effects in addition to their cholesterol-lowering action; these include decreasing fibrinogen (and blood/plasma viscosity), decreasing tissue factor expression on macrophages, decreasing platelet aggregation and deposition, and increasing fibrinolysis (by reducing PAI-1 levels)(59). There seem to be some differences among the various statins on the market, however (59); the clinical significance of these differences is uncertain, and they lower CRP to the same extent.

Since both aspirin and statins have multiple actions that may prevent atherosclerotic disease, it is obviously impossible to know what portion of their beneficial action is due to inhibiting inflammation.

**CRP and Cardiovascular Risk**



**Approximate ranges of CRP concentrations in different cardiovascular conditions**

The published studies suggest the following schema:

- Many years before clinically apparent cardiovascular disease presents, inflammation may occur in coronary and peripheral vessels. The inciting stimulus is not known; some studies point to an important role for infectious agents such as *C. pneumoniae* and/or CMV. Inflammation or infection in non-vascular sites can also increase CRP and other acute phase reactants. The preventive efficacy of aspirin and statins may be due, in part, to their ability to prevent both local inflammation and acute phase responses.
- In some individuals, the intracoronary inflammatory process is particularly active. These individuals may be more likely to develop unstable angina. In patients with UA, CRP levels correlate rather well with adverse events such as myocardial infarction and death. There is good evidence that the inflammatory process in these patients is within the coronary vessels, not in the myocardium, since transient myocardial ischemia does not elevate measures of acute inflammation.
- Myocardial infarction (necrosis) does trigger inflammation within the damaged tissue; IL-6 is produced in myocytes at the viable border zone of the infarct (60) and the blood concentrations of IL-6, IL-8, CRP, SAA, and leukocytes increase markedly during the 24 – 48 hour period after infarction (61). CRP levels correlate quite well with the extent of myocardial damage and also (inversely) with ejection fraction. CRP can bind to cardiac muscle fibers and activate complement, possibly contributing to local leukocyte infiltration, further myocardial injury, and infarct extension.

**Using CRP blood levels in clinical practice**

I think the most persuasive findings are those that associate a **low** CRP with low risk of developing CAD in apparently healthy adults. Similarly, in patients with unstable angina, having both a low CRP and a low troponin I level evidently identifies a group that has very low risk of progression to MI, need for revascularization, or death. Perhaps this information can be useful for establishing prognosis, reassuring patients, etc. At the other extreme, a high CRP level in a patient with unstable angina or myocardial infarction should heighten one's concern for subsequent adverse events; the existing data do not suggest which interventions should be based on this finding, however.

One problem derives from the way the studies have been done: in each study reported to date, the definition of a "low" and "high" CRP has been determined by dividing a large population into segments (tertiles, quintiles, etc.), using the assay performed in a single lab. Although the "cut-off" value for a high test has not

differed markedly from population/lab to population/lab, there has also not been impressive uniformity – and a cutoff set at 1.5 mg/L would include a lot more patients than one set at 3 mg/L. Practicing physicians may have difficulty knowing how to interpret the results of CRP assays until the range of values in the patient's population/lab has been established. Nevertheless, "high sensitivity" CRP measurement is now widely available in the U.S. and elsewhere, including the Parkland Clinical Chemistry Laboratory, and the assay has been approved by the F.D.A. for assessment of cardiovascular risk. Suggested guidelines endorsed by Dr. I. Jialal, Director of the Parkland lab, are indicated in the Table.

**Population distribution of hs-CRP in apparently healthy American men and women**

| Quintile | Range (mg/L) | Risk estimate |
|---|---|---|
| 1 | 0.1 – 0.69 | Low |
| 2 | 0.7 – 1.1 | Mild |
| 3 | 1.2 – 1.9 | Moderate |
| 4 | 2.0 – 3.8 | High |
| 5 | 3.9 - 15 | Highest |

Although some of the studies suggest that a high CRP (or SAA, or IL-6) level can identify patients at high risk of CAD despite normal (or even low) total cholesterol levels, randomized prospective clinical trials are required now to determine whether intervention (e.g., with ASA or statin) can reduce both CRP and CAD in such individuals.

Some of the remaining questions:

- How often (or how many times) should CRP be measured in a healthy adult in order to establish his/her risk category?
- What is the best "cut point" for defining increased CAD risk in different patient populations – for each age group, gender, ethnic group, etc.?
- Does measuring other risk factors (e.g., fibrinogen or von Willebrand factor) increase the ability to predict CAD risk sufficiently to warrant measuring these blood levels in addition to CRP?
- Is a high CRP an indication for aspirin or statin treatment in someone who has low total cholesterol? (and what is "low" in this context?) What if the patient's total cholesterol/HDL ratio is less than 5?
- Should certain patients be followed with serial CRP measurements to try to anticipate the occurrence of myocardial ischemic events? If so, which patients? How often? And what therapeutic or prophylactic response should be taken?
- Should anti-inflammatory therapy be part of the routine management of MI with elevated CRP? If so, with what?
- What is the value of measuring CRP in patients with known CAD who are already taking a statin and/or aspirin?

**The Biological Roles of C-Reactive Protein.**

Why do we have CRP? It is found in invertebrates that don't have antibodies or complement, and it has been highly conserved through vertebrate evolution. What are its functions?

CRP has five non-covalently-linked subunits. In electron micrographs, it has a planar pentagonal appearance; it is a member of the pentraxin family, which includes serum amyloid P and other proteins. Its molecular mass is approximately 118 kDa. Its crystal structure was recently reported (62). Its major known ligand is phosphocholine.

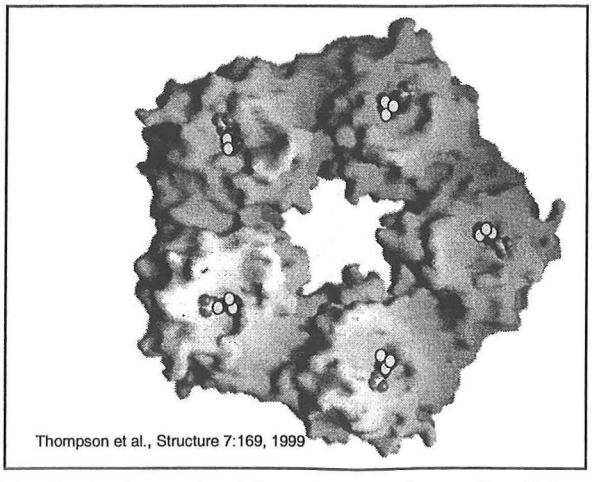

Thompson et al., Structure 7:169, 1999

Figure (right): structure of CRP with 5 molecules of bound phosphocholine.

The CRP gene has been mapped to chromosome 1q21. A single nucleotide polymorphism with allele frequency in Caucasians of 0.11 was recently reported (63).

*Anti-infective actions:*

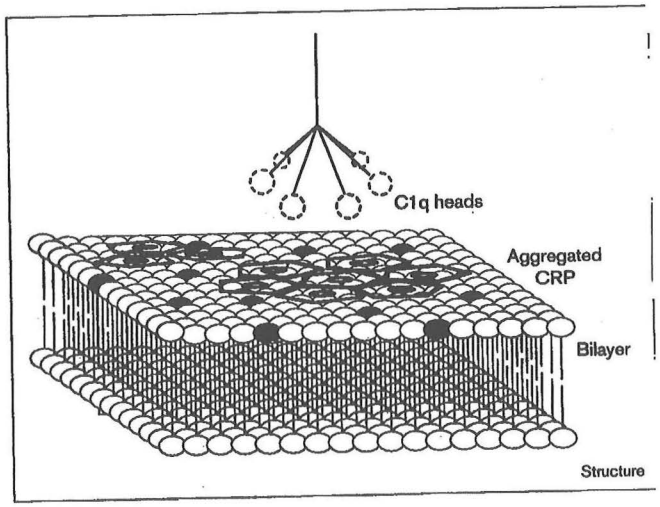
Although most work on the anti-infective role of CRP has focused on immunity to *S. pneumoniae*, CRP is also thought to be involved in protecting animals from infection with microbes as diverse as *Haemophilus influenzae* and *Leishmania.*

CRP protects mice from lethal pneumococcal infection (64,65). Serum bactericidal activity toward *Haemophilus influenzae* is also mediated by CRP, which binds to a phosphorylcholine moiety on the Haemophilus cell surface lipopolysaccharide. Variability in the expression of phosphorylcholine may influence the pathogenesis of *Haemophilus influenzae* infections (66). Similarly, the LPS of commensal Neisseria is substituted with phosphocholine while that of *Neisseria meningitidis* is not (67). It appears that CRP may restrict tissue invasion by these human pathogens, all of which inhabit the oropharynx, to those bacteria that do not express LPS-linked phosphocholine. The major binding receptor for CRP on leukocytes is Fcγ receptor IIA (68). CRP binding is allele specific: R131 binds with high avidity, H131does not (69). (Interestingly, H131 preferentially binds $IgG_2$, the major polysaccharide-binding immunoglobulin. Individuals with the R131 allele are at increased risk of invasive meningococcal and pneumococcal disease (70,71), in keeping with the notions that (a) anti-polysaccharide antibodies are less effective at promoting phagocytosis when their high affinity Fc receptor is absent , and (b) CRP doesn't provide effective "back-up" opsonization since theinvasive bacteria don't express phosphocholine.

Like some immunoglobulins, CRP can activate complement via the classical pathway (C1q) and it can opsonize particles for phagocytosis. Aggregation of CRP molecules is required for C1q binding (Figure). CRP is thought to bind to a surface of eukaryotic only after conversion of some of the phospholipids to lysophospholipids (black balls) by $PLA_2$.

Summary: CRP behaves very much like a *broad-specificity antibody.* It has been called an "ante" antibody, since it has been found in invertebrates that do not make either immunoglobulins or complement. It may also be considered a "pattern recognition" molecule: its "pattern" seems to be determined by the configuration of phosphocholine moieties on membranes.

Other APP pattern-recognition molecules include mannose-binding protein (which can also activate complement) and LPS-binding protein.



Thompson et al. Structure 7:169, 1999

*Anti-inflammatory actions (72):*

In keeping with the notion that the acute phase response prevents systemic inflammation (see below), CRP has several anti-inflammatory actions. Although most of these have been detected in vitro, in vivo evidence for its anti-inflammatory role also exists. For example, transgenic mice that express rabbit CRP are protected from the lethal reaction to endotoxin, IL-1β + TNF-α, and platelet activating factor (PAF) (73). (CRP binds and neutralizes PAF.)

In vitro, recent studies have found that CRP stimulates sIL-6R shedding from cells (74). (sIL-6R binds IL-6 and prolongs its half-life in plasma.) CRP also stimulates IL-1Ra release from monocytes (75,76). Other studies have found that CRP increases L-selectin shedding from neutrophils and prevents neutrophil-endothelial cell adhesion (77).

Other anti-inflammatory APPs include IL-1Ra (78), sIL-6R, $\alpha_1$-acid glycoprotein, and soluble TNF receptors. The numerous antiprotease and antioxidant APPs may also be considered anti-inflammatory, since they help confine the impact of potentially toxic proteases and oxidants to local sites of inflammation.

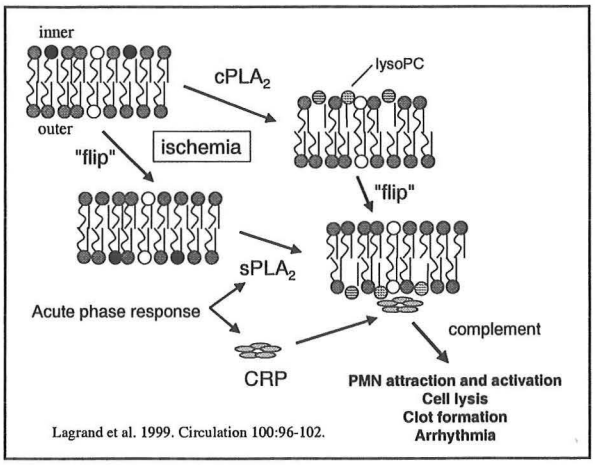
*Procoagulant actions*

Recent findings point to IL-6 as the major procoagulant cytokine in humans and chimpanzees (79,80). It is thus not very surprising that CRP, which is produced in response to IL-6, is able to increase the expression of tissue factor on monocytes (81). Other procoagulant APPs include fibrinogen and plasminogen activator inhibitor-1. Although clotting plays an important role in walling off infection at local sites (animals given anticoagulants don't develop abscesses), a protective function of these systemic procoagulant activities is not apparent.

*Scavenging actions*

Although CRP does not bind to normal cell membranes, it can bind avidly to cells that are undergoing apoptosis or necrosis, possibly because it can recognize the lysophosphatidylcholine (lysoPC) that appears on the surfaces of dying cells. It then binds and activates complement (C1q), initiating a local inflammatory reaction that attracts neutrophils and monocytes to the scene. CRP also binds nuclear ribonucleoproteins and histones (thus, chromatin) under physiological extracellular ionic conditions, suggesting that it may be involved in scavenging nuclear antigens (82) and enhancing chromatin clearance from the blood into liver and spleen (83).

Hack et al. have proposed a model to account for the ability of CRP to bind to damaged tissues and contribute to tissue recovery (84)(see Figure at right)(85). They note that, when a cell is injured, the lipids in its plasma membrane rearrange so that some of the phospholipids that normally face the cytosol get "flipped" to the outside. They suggest that that secretory phospholipase $A_2$ ($sPLA_2$, another acute phase protein) can act on the phosphatidylcholine molecules in the outer leaflet of the plasma membrane after this flipping has occurred. Alternatively, cytosolic phospholipase $A_2$ ($cPLA_2$) might act on the membrane prior to flipping. LysoPC can be bound by CRP, which activates complement at the site, attracting phagocytes that remove the damaged cells. The complement activation could be autocatalytic, since the membrane-perturbing action of C9 would cause further phospholipid "flipping", allowing $sPLA_2$ to generate more lysoPC, etc. CRP's ability to bind phosphocholine may also prevent autoantibodies from forming to host lipids or the proteins adjacent to them in the membrane.




**Serum amyloid protein** (SAP) also may play a role in remodeling damaged tissues. It is present in human serum (30 - 50 mg/L). It shares considerable sequence identity with CRP and, like CRP, it is a pentraxin (86). It undergoes calcium-dependent binding to chromatin and native DNA (87), fibronectin, glycosaminoglycans, and C4-binding protein. SAP is also found in amyloid deposits, including those of Alzheimer's disease. SAP is not an acute phase protein in humans; in mice, where its concentration increases > 10-fold during the APR, it is associated with clearance of chromatin from the circulation, shifting the distribution of chromatin from the spleen and kidney to the liver (83). SAP knockout mice develop a lupus-like syndrome. (It's interesting that human patients with systemic lupus erythematosus typically don't produce CRP when their disease is active.)

**Summary:** CRP is a dynamic component of the systemic response to infection, injury, and other stresses. There is very little evidence that it is pro-inflammatory. Rather, like other APPs (see below), it has anti-infective, procoagulant and anti-inflammatory actions that promote host defense and tissue repair. Its ability to stimulate monocyte tissue factor expression could possibly promote atherosclerosis (thrombosis), while its role in scavenging chromatin and clearing dead cells could account for its reported ability to exacerbate myocardial necrosis.

**CRP: The Eyes of the Hippopotamus**

CRP and SAA are the most dynamic of the acute phase proteins; their concentrations can increase 1000-fold or more in response to various stimuli. It seems likely that, in addition to whatever direct role CRP may play in the pathogenesis of atherosclerosis, it is also a very sensitive marker for the body's systemic responses to local inflammation. In the African metaphor, it's the eyes of the hippopotamus – much more of the beast lies below the surface.

**The Acute Phase Response**

The term "acute phase response" (APR) refers to the body's systemic adaptations to injury or infection (88). Stimuli that can trigger the APR include bacterial infection, trauma, neoplasms, bone fracture, tissue infarction, immunological reactions and diseases, and childbirth. Broadly defined, the APR is regulated by the *hypothalamic-pituitary-adrenal axis*, the *sympathetic-adrenomedullary axis*, and cytokine-induced changes in protein synthesis. Its impact is broad: bone formation decreases, hematopoiesis decreases, nitrogen balance becomes negative, etc. In its more common usage, the term "acute phase response" refers to the changes that occur in the blood (or intracellular) concentrations of several proteins. These proteins are sometimes called "*acute phase reactants*" or "*acute phase proteins*" (**APPs**). The broader definition will be used here. The term "acute" is obviously inaccurate when the same systemic responses persist for many years.

The APR has been conserved from higher invertebrates to humans. It existed before animals had evolved immunoglobulins, lymphocytes, or complement. It is thus the most primitive of the known host defense mechanisms. Only recently has it become obvious that the body uses many of the same molecules (ACTH, cortisol, catecholamines, IL-6, others) to regulate its systemic adaptations to infection, injury, exercise, and other stresses.

**APPs.** The "positive" APPs are those that become more abundant in the blood during the APR. The concentrations of some of these proteins increase as much as 50%, others may increase from 2- to 5-fold, while others may increase 1000-fold or more (see Table 1). The concentrations of the "negative" APPs decrease; most prominent among these is serum albumin, although decreases in transthyretin and apolipoprotein AI can also be physiologically important. The following Table is modified slightly from reference (89).

| | Increase by | | Normal plasma concentration (mg/L) | Presumed functions |
|---|---|---|---|---|
| **Positive APPs** | Up to 50% | Ceruloplasmin | 150 – 600 | Copper transport; oxidant |
| | | Complement C3 | 800 – 1700 | Opsonizing, bactericidal |
| | | Complement C4 | 150 - 650 | Classical pathway |
| | Up to 5-fold | Fibrinogen | 2000 – 4500 | Procoagulant |
| | | Plasminogen activator inhibitor – 1 | | Procoagulant |
| | | Haptoglobin | 400 – 1800 | Scavenge hemoglobin |
| | | α1-protease inhibitor | 2000 – 4000 | Inhibit serine proteases |
| | | α1-antichymotrypsin | 300 – 1600 | Inhibit serine proteases |
| | | α1-acid glycoprotein | 550 – 1400 | Uncertain (anti-inflammatory) |
| | 5- to ~100-fold | Mannose binding protein | | Microbial pattern recognition; complement activation |
| | | LPS binding protein | ~ 5 | Microbial pattern recognition; opsonizing |
| | | Phospholipase $A_2$ (secretory) | < 0.250 | Uncertain (remodeling damaged membranes? anti-staphylococcal activity?) |
| | Over 1000-fold | CRP | < 2 | See text |
| | | Serum amyloid A | < 10 | See text |
| **Negative APPs** | | Albumin | | |
| | | Transthyretin | | Thyroid hormone transport |
| | | Transferrin | | In humans |
| | | Apolipoprotein A | | HDL constituent |

Other positive APPs: ferritin, other complement components, von Willebrand factor.

**The Acute Phase Response: presumed functions**

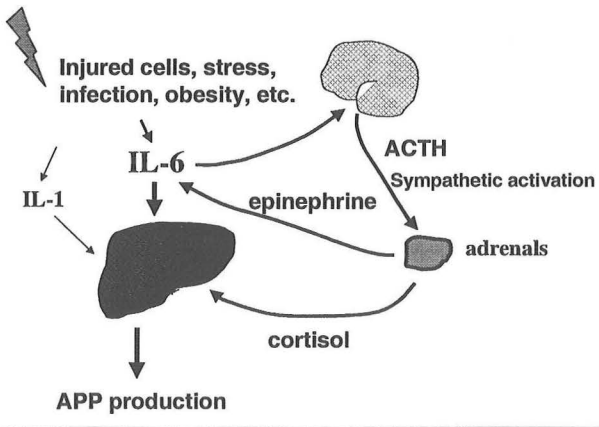
| Category | Function | Mediators | Comments |
|---|---|---|---|
| **Anti-infective** | Recognize invading microbes | LBP, MBP, CRP, others | "Pattern recognition" molecules and/or their receptors |
| | Mobilize leukocytes into the circulation | cortisol, epinephrine; G-CSF, M-CSF | Release marginated PMN; stimulate myelopoiesis. |
| | Target leukocytes to inflamed local sites | cortisol, IL-10, epinephrine, CRP | Prevent non-specific leukocyte-endothelial adhesion, so neutrophils can home to tissues with activated endothelium. |
| | Kill invading microbes | Complement; $sPLA_2$ | $sPLA_2$ has anti-staphylococcal and anti-streptococcal activity |
| | Prevent microbial growth | iron, zinc, sequestration; fever | Lactoferrin binds and sequesters iron; metallothionein sequesters zinc. Many microbes grow more slowly at supraphysiological temperatures. |
| **Anti-inflammatory** | Prevent leukocyte-endothelial adhesion to vessels outside inflamed local sites | cortisol, epinephrine, IL-10, CRP | Limit inflammation to local site(s) |
| | Neutralize toxic molecules released from inflamed local sites | IL-1Ra, soluble TNF receptors; protease inhibitors, anti-oxidants, PAF | APPs include metallothionein, hemopexin, numerous protease inhibitors, CRP |
| | Prevent secretion of inflammatory mediators by circulating leukocytes | IL-10, α-MSH, cortisol, ? | "Reprogramming" of leukocyte responses to bacterial agonists |
| **Procoagulant** | Wall off injured/inflamed sites | | Fibrin deposition is required for abscess formation and delayed hypersensitivity reactions. Epinephrine has anti-coagulant activity; TNF-α is pro-fibrinolytic. tPA is also an acute phase reactant. |
| | Promote clotting | fibrinogen; monocyte tissue factor expression | |
| | Inhibit fibrinolysis | PAI-1 | |
| **Scavenging** | Remove dead cells, debri, intracellular constituents (hemoglobin, chromatin, ribonucleoprotein); avoid autoantibody formation | Macrophage phagocytosis of apoptotic or necrotic cells; CRP or SAP + complement; haptoglobin | Phospholipase $A_2$ may assist scavenging function by preparing cell membranes for CRP binding (see text) |
| **Energy redistributing** | Shift from exogenous to endogenous nutrient sources ("our genome is designed to fight against infection with minimal food intakes" (Fernandez-Real and Ricart, 1999). | Mobilize glucose, NEFA, triglycerides, glutamine; decrease albumin synthesis. | Energy supply shifts from muscle to brain, immune cells. Glucose availability favored by insulin resistance, glycogenolysis, gluconeogenesis. NEFA mobilized by increased lipolysis. Decreased albumin synthesis favors increased APP production. |

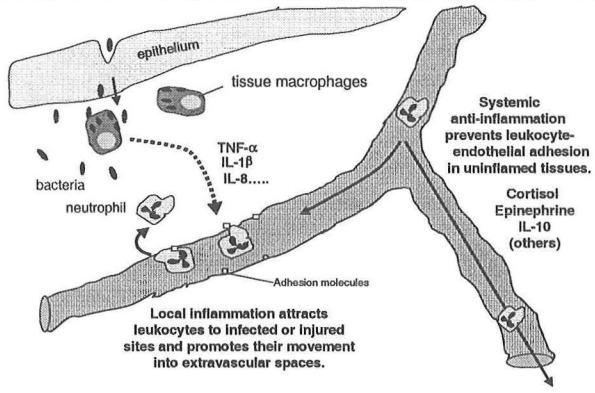
Summary: the APR is a highly integrated set of physiological changes that *promote effective local immune responses*, thus preventing the dissemination of microbes within the body, while *preventing systemic inflammation* and the damage it might cause (see drawing on the next page). While the APR was doubtless very important for primitive human hunter-gatherers (fight/ flight/ defense vs. microbial invaders), many of its components are

probably pro-atherogenic (see below). As it has become possible for humans to live beyond age 40 - 50, the impact of prolonged, low level APRs has become evident. Indeed, the APR has been invoked to account (in part) for the frailty of aging, dementia, and other modern disabilities (90).

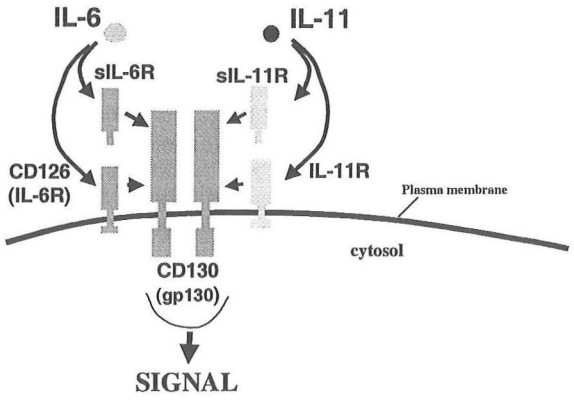
**Regulation of acute phase protein production**

The most prominent stimulus for acute phase protein production is a cytokine, **interleukin-6** (IL-6). IL-6 is made by most cells, which release it when they are injured; at least in rodents, β-adrenergic agonists also trigger cells to release IL-6. Since mice that lack IL-6 have exaggerated inflammatory responses to various stimuli and are unable to control inflammation at local sites of infection, this cytokine is now thought to have predominantly anti-inflammatory or immunomodulatory actions.(72). It is also attractive to think of IL-6 as an "SOS" cytokine that mobilizes systemic responses to injury or infection: in addition to its role in triggering acute phase protein production, it is a potent activator of the hypothalamic-pituitary-adrenal axis. (This link may increase acute phase protein production, since moderate concentrations of cortisol are permissive for APP production. Higher cortisol concentrations are inhibitory. In vivo, endogenous cortisol inhibits TNF-α and IL-1β production at lower concentrations than are required to inhibit IL-6 (91).) Other cytokines, particularly IL-1, also play roles in APP regulation; the importance of IL-6 likely relates to its production by most cells, its broad stimulatory activity toward APP genes, and its ability to bind in blood to the soluble IL-6 receptor (sIL-6R), which prolongs its half-life and enhances its potency. The IL-6 receptor (IL-6R) has 2 components, one of which (CD130) is shared by the receptors for IL-11, LIF, oncostatin-M, and CNTF. Of these molecules, IL-6 is the dominant inducer of APP.




**Systemic anti-inflammatory responses favor neutrophil homing to inflamed tissues.** Bacteria that invade through a break in the epithelium trigger tissue cells (macrophages and others) to release pro-inflammatory mediators, such as TNF-α and IL-1β; these mediators act on local vessels to increase adhesion molecule expression and increase capillary permeability. Chemokines (e.g., IL-8) attract neutrophils to vessels within the inflamed tissue, where increased expression of adhesion molecules on endothelial cells allows them to stick to the vessel wall. Neutrophil diapedesis into the extravascular space follows. The systemic response is mediated by cortisol, epinephrine, IL-10, CRP and other molecules; by preventing neutrophil adherence to vessels outside the inflamed tissue, it promotes local inflammation and microbial killing while preventing injury to distant organs.



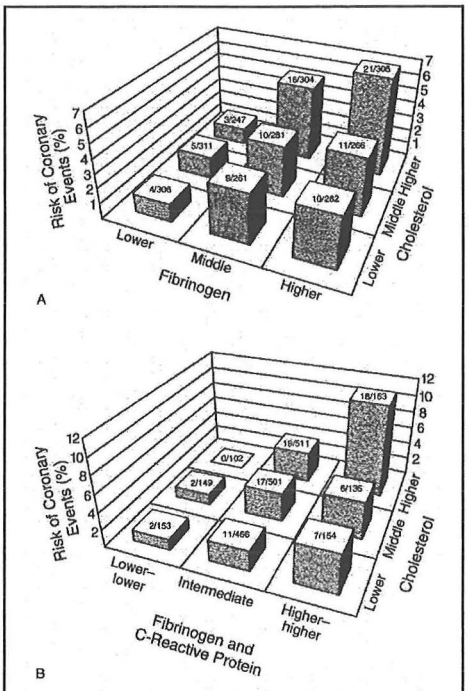

(This summary ignores many details. The production of different APPs is regulated independently; in actuality, "..the APR is not a single, coordinate, global phenomenon, but rather...the integrated sum of multiple, separately regulated changes in gene expression "(88). Many other mediators, particularly IL-1, interact with IL-6 to regulate APP production. See Kushner's accounts for a more detailed and accurate description (88,92).)

In many situations, CRP levels seem to reflect (amplify) the IL-6 signal. Elevated IL-6 levels occur following myocardial infarction (61)

and during unstable angina (24). A decrease in plasma IL-6 during the first 48 hours of hospital admission for unstable angina was associated with an uneventful hospital course, whereas an increase occurred in most patients who had cardiac events (24). In apparently healthy men, baseline IL-6 levels predicted risk of subsequent MI; although there was a strong correlation between IL-6 and CRP levels, the relationship of IL-6 with subsequent risk remained after controlling for CRP (38). Although measuring both IL-6 and CRP might provide prognostic information that exceeds that obtained from CRP levels alone, much more study is needed.

**Acute phase responses that may contribute to an atherogenic "profile":**

**1. Procoagulant changes.** ***Fibrinogen*** is the major coagulation protein in the blood; it also strongly affects blood viscosity and the erythrocyte sedimentation rate (ESR). In their meta-analysis of 12 population-based studies of CAD risk, Danesh et al. found a summary odds ratio of 1.8 (95% CI, 1.6-2.0) for individuals in the upper third of the fibrinogen concentration distribution compared to those in the lowest third (44) (in the same meta-analysis, the odds ratio for CRP was 1.7). Fibrinogen and CRP levels usually correlate strongly with each other (13,15,93). In the ECAT study, Thompson et al. found that fibrinogen and CRP were good predictors of coronary events in adults with CAD, even in individuals with low total cholesterol levels (Figure). Even more striking was the ability of a low CRP + low fibrinogen to predict event-free survival in individuals with high total cholesterol!



Thompson et al. 2000. NEJM 332:635

Fibrinogen also contributes importantly to blood viscosity. A study from Germany found peak values in the winter months for plasma viscosity, platelet count, fibrinogen, PAI-1, LDL cholesterol and triglyceride levels; HDL cholesterol and cortisol levels were maximal in the summertime (94). Cardiovascular mortality increases by ~30% in the wintertime.

Another important APP is ***plasminogen activator inhibitor-1*** (PAI-1). It is perhaps the most important anti-fibrinolytic protein in plasma. Importantly, **tissue plasminogen activator** levels also increase during the APR; perhaps the increase in tPA is a compensation for higher PAI-1 levels. In any case, in studies in healthy adults, levels of tPA have also correlated with (a) CRP and (b) CAD risk (95).

Blood levels of von Willebrand factor, another APP, also predict CAD risk; in some studies this risk prediction is independent of, and stronger than, that associated with CRP (96). It is uncertain whether high vWf concentrations reflect the APR or diffuse endothelial activation, however; Jager et al. favor the latter (96).

**2. Elevated $sPLA_2$.** Secretory nonpancreatic type II phospholipase $A_2$ ($sPLA_2$) is not only expressed in atherosclerotic arterial walls, but it is an APP; circulating levels can increase >100-fold during the APR. In addition to having antibacterial actions (it is the most potent anti-staphylococcal and anti-streptococcal molecule in acute phase plasma (97)), it also is involved in remodeling HDL and LDL and damaged membranes (see above). $sPLA_2$ binds to decorin, a collagen-associated proteoglycan, and possibly contributes to LDL modification in the arterial wall (98). A recent study in Japan found that $sPLA_2$ was superior to CRP for predicting subsequent cardiac events in a population of patients who underwent cardiac catheterization for chest pain or ischemic EKG changes (99).

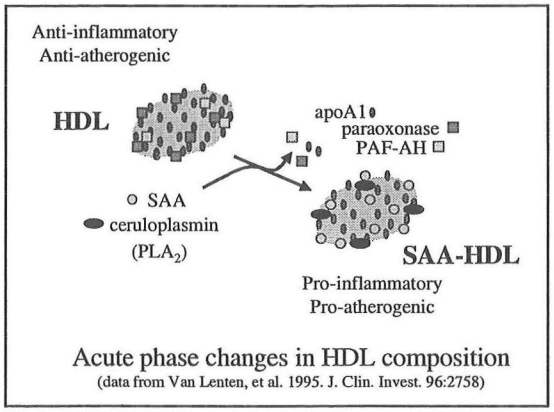
**3. Dyslipidemia and Serum Amyloid A (SAA)**

*a. Hypertriglyceridemia.* Triglyceride and VLDL levels increase during the APR, and hypertriglyceridemia has been associated with increased risk of CAD in recent studies (100). These changes are often attributed to decreases in lipoprotein lipase (LPL) and hepatic lipase, although recent studies in mice indicate that LPS-induced hypertriglyceridemia can be blocked (by an antibody to TNF-$\alpha$) without affecting muscle or adipose tissue LPL activity (101). Free fatty acid concentrations also rise, due to increased lipolysis in adipose tissue and increased hepatic fatty acid synthesis. See (102) for a more complete review of these changes.

*b. Changes in HDL concentration and composition.* Two related phenomena seem important:

- *Decreased circulating HDL-cholesterol concentration.* In some studies of healthy individuals, CRP has had significant negative correlations with apoA-I and apo-AII (36). Other studies have not found such correlations (42,48), but these studies did find inverse correlations between CRP and HDL-cholesterol (48). There is little doubt that acute phase responses are usually associated with lower HDL-cholesterol levels (40).
- *HDL remodeling.* Early in the APR, HDL particles acquire triglyceride (103); this may reflect remodeling due to secretory phospholipase $A_2$ (104). Later they undergo even further remodeling due to serum amyloid A. During the APR, serum levels of **serum amyloid A** (SAA) may increase 500- to 1000-fold. SAA is carried almost entirely on $HDL_3$; when it binds to $HDL_3$ it displaces apoA-I. Other acute phase changes in HDL composition include decreases in cholesterol (105,106), platelet activating factor acetylhydrolase (PAF-AH)(107), and paraoxonase (which prevents LDL oxidation) (107). The anti-oxidant and anti-inflammatory character of the particles is diminished as they lose PAF-AH and paraoxonase. In addition, HDL gains ceruloplasmin, another acute phase protein, and becomes more pro-oxidant.

**Serum amyloid P** (SAP): scavenges chromatin. An APP in mice, not in humans. A pentraxin (like CRP). Found in Alzheimer's plaques, most amyloid deposits. **Serum amyloid A** (SAA): Mr = 12,000, binds HDL, alters its properties. APP in human, mouse. Several isoforms. Contributes to *secondary (AA) amyloidosis.* (*Primary amyloidosis (AL)* is due to deposition of immunoglobulin light chains.)



Acute phase changes in HDL composition
(data from Van Lenten, et al. 1995. J. Clin. Invest. 96:2758)

These changes are associated with more rapid clearance of HDL from the circulation (108); this seems to be the major mechanism by which the circulating HDL concentration decreases. Among the potential explanations for this enhanced clearance is the observation that SAA-HDL has a higher affinity for binding macrophages than does HDL (106,109).

When compared with normal HDL *in vitro*, acute phase (SAA-)HDL surrenders cholesterol esters more rapidly to macrophages and, at least in some studies, is also significantly less able to enable cholesterol efflux from these cells (109). Replacement of more than half of the apoA-I by SAA may be required for these changes, however.

In summary, acute phase HDL is *pro-inflammatory* and *pro-atherogenic (see Figure).*

(Deposition of an amino-terminal fragment of SAA in macrophage-rich tissues is the basis for *secondary amyloidosis*, an incurable process that may complicate chronic inflammatory conditions such as rheumatoid arthritis.)

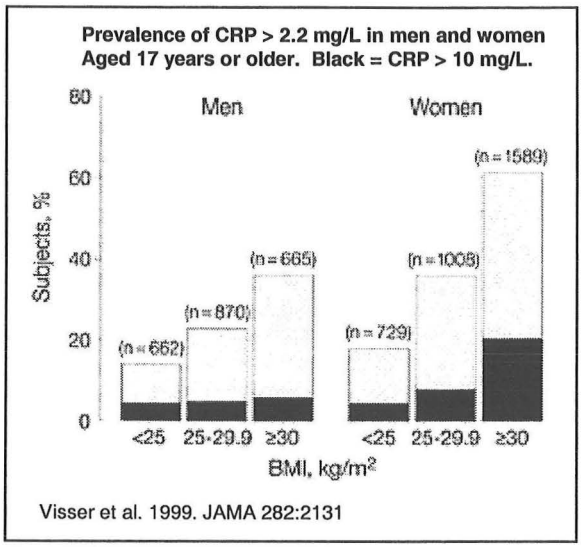
**4. Leukocytosis.** Although leukocytosis is not usually included in discussions of the APR, it may result indirectly from the actions of cortisol (which promotes demargination of leukocytes from postcapillary venules (110)) and CRP (which may induce L-selectin shedding from leukocyte surfaces, thus interfering with leukocyte adhesion), as well as from marrow stimulation by M-CSF and G-CSF (both of which increase during acute phase responses). In their 1998 meta-analysis, Danesh et al. found 19 prospective studies of leukocyte count and CAD. The 7 largest studies all adjusted for smoking, which increases blood leukocyte counts (as do diabetes mellitus and age (93)). Individuals in the top third (mean = 8.4 x $10^9$/L) had a risk ratio of 1.4 (95% CI, 1.3-1.5) relative to those in the bottom third (mean = 5.6 x $10^9$/L). The different cell types were not specified.

**Acute phase responses that have been associated with increased risk of coronary vascular disease in one or more studies:**
high CRP
high fibrinogen
high tPA
high von Willebrand factor
leukocytosis
high triglycerides
low HDL-cholesterol
low apoA-I
low albumin
high $PLA_2$
high ferritin

**5. Hypoalbuminemia.** Although hypoalbuminemia not a likely atherogenic factor itself, a recent metaanalysis found that the serum

albumin concentration has a statistically significant inverse association with CAD (44). Low albumin levels correlated with age, smoking, obesity, and blood pressure.

*Summary: a list of the known laboratory-based risk factors for CAD includes many acute phase proteins (boxon previous page). Could activation of the APR be the common mechanism by which smoking, obesity, stress, etc., contribute to atherosclerosis?*



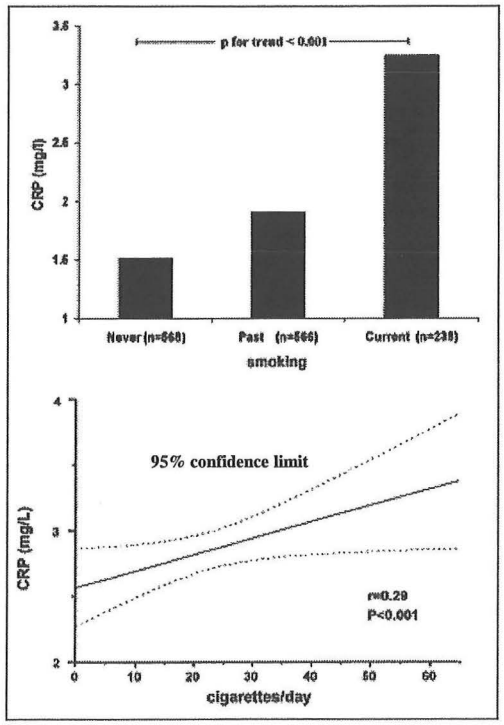
Visser et al. 1999. JAMA 282:2131

**Obesity** (see Dr. Nicola Abate's Grand Rounds of January 6, 2000). There is a strong correlation between BMI and CRP levels, particularly in women (111) but also in men (36,42). In the study by Visser et al. (Figure), the prevalence of "clinically elevated" CRP (> 10 mg/L) was 4.0% in normal-weight women, 7.7% in overweight women (BMI 25 – 29 kg/m$^2$), and 20.2% in obese women (BMI > 30 kg/m$^2$). Older women were less likely to have elevated or clinically elevated CRP than younger women. CRP levels also correlate directly with the waist/hip ratio.

BMI is also strongly associated with plasma leptin levels (112,113). A relationship between sleep apnea, visceral obesity, insulin resistance, and hypercytokinemia (IL-6, leptin) has been proposed (113).

**Age.** In apparently healthy men, CRP levels increase with age (36). Similar results were found for both genders in another study (114). The means were 1.90 mg/L in the 25-34 year age group vs. 3.03 in the 75-84 year group (114).

**Smoking.** Smokers have significantly elevated CRP levels (again, these are within the traditional "normal" range – below 10 mg/L)(36,42). In some studies, smoking has had such a striking effect on CRP that smokers have been considered as a separate group: even when this has been done, CRP has remained an independent risk factor for CAD. Smoking may trigger APR by inducing low-grade airway inflammation, predisposing to chronic bronchitis, or other mechanisms. Cigarette smoke contains bacterial endotoxin (115); one recent report found an association between high levels of circulating endotoxin and carotid and coronary atherosclerosis in smokers (116). See Figure at right.



CRP levels are elevated in smokers. Rohde.1999. Am.J.Cardiol. 84:1018

**Non-insulin-dependent (type 2) diabetes mellitus.** Reaven (117) suggested that the increased risk of CAD in hypertensive patients, and the fact that this risk is not reduced with antihypertensive treatement, is due to the clustering of hypertension, hyperinsulinemia (insulin resistance), hypertriglyceridemia, and low HDL-cholesterol. More recently, pilot studies have suggested an association between insulin resistance and CRP levels in healthy individuals (118). Pickup et al. (119,120) and Fernandez-Real and Ricart (121) have suggested that the APR makes an important contribution to NIDDM and, in addition, to metabolic syndrome X (obesity, NIDDM, hypertension, atherosclerosis). Blood abnormalities which are present in both acute phase responses and metabolic syndrome X include hyperinsulinemia, hypertriglyceridemia, high leptin, low HDL, high CRP, high complement and SAA, high fibrinogen, high PAI-1 and von Willebrand factor, high cortisol, low testosterone and zinc (119). Elevated levels of ferritin, another APP, may also be part of the "insulin

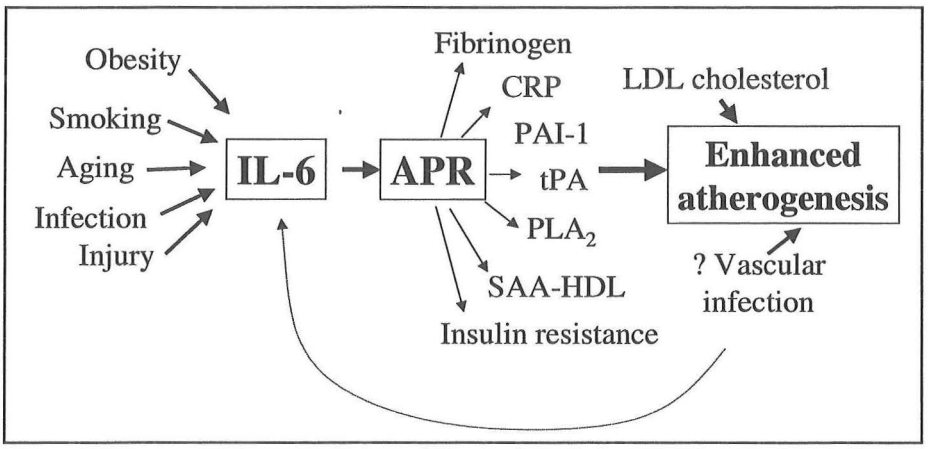
resistance syndrome" (122). In obese individuals, hyperleptinemia is associated with high IL-6 and cortisol levels in addition to its strong correlation with BMI (123).

Summary: there is intriguing (but still inconclusive) evidence that activation of the APR plays a role in the atherogenesis associated with obesity, smoking, age, and NIDDM.

**Obesity, smoking, inflammation, stress: is there a common pathway to atherothrombotic disease?**

Since visceral obesity, smoking, age, and NIDDM are all associated with both low-grade acute phase responses and increased risk of coronary disease, and since many of the changes that occur during acute phase responses are pro-atherogenic, is the APR the link that connects these diverse conditions to atherosclerotic cardiovascular disease? And, if so, what is the "signal" that triggers the APR in each of these conditions?

Is there a common stimulus that provokes the APR in patients with the diverse conditions mentioned above? Yudkin (124) has proposed that **IL-6** is the mediator that links the APR to visceral obesity, insulin resistance and CAD ("metabolic syndrome X"). A similar hypothesis has been suggested by McCarty (125).



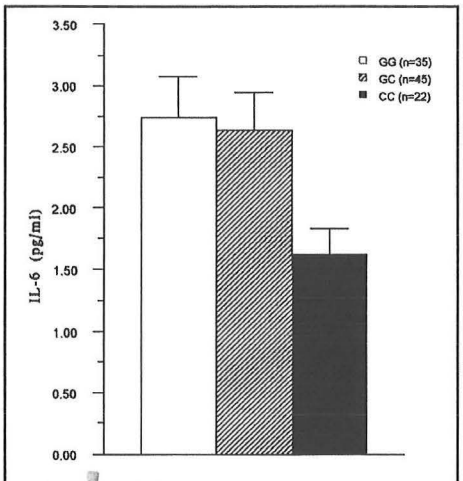

A summary of the evidence:

- IL-6 is a major trigger for the APR. This conclusion is supported by the observed consequences of gene disruption experiments in mice: IL-6 -/- mice do not mount an acute phase response (126), whereas TNF-α -/- mice are essentially normal in this regard (127). (IL-1 KO mice also do not have a normal APR, pointing to important interactions between IL-1 and IL-6 in APR activation.)
- IL-6 is also a major stimulus for activation of the hypothalamic-pituitary-adrenal axis.
- In numerous studies of chronically ill patients, there has been a tight correlation between CRP and IL-6 levels, but not between CRP and levels of other cytokines.
- In several population-based studies, blood IL-6 concentrations have predicted total and cardiovascular mortality (39-41); in one study, this association was stronger than, but additive to, that for CRP (39).
- Many conditions that predispose to CAD are associated with elevated blood IL-6 levels:
    - Age
    - Smoking
    - Obesity
    - Diabetes mellitus (NIDDM)
    - Chronic inflammation
    - Stress
- How obesity might produce elevated blood IL-6 levels may be explained by the striking observation that approximately 10% to 35% of the body's IL-6 is produced in adipose tissue (128,129). Omental adipose tissue evidently produces significantly more IL-6 than subcutaneous adipose tissue (129), in keeping with the observation that waist-to-hip ratio is an independent risk factor, independent of BMI, for an elevated blood CRP (111) and the "metabolic syndrome." Adipose tissue also produces and releases TNF-α (130); one study found that blood TNF-α levels

correlated with waist-to-hip ratio, but not to BMI (130). Since TNF-α can trigger IL-6 release, one theory holds that it is adipose tissue TNF-α that actually is the "driver" behind the visceral obesity-APR-CAD connection (125). Evidence against this hypothesis is the finding that subcutaneous adipose tissue evidently releases IL-6, but not TNF-α, in vivo (128). On the other hand, blood levels of soluble TNF-α receptor (sTNF-R55, an indicator of TNF-α activation), correlate with blood leptin levels in both controls and diabetic subjects (112).

The IL-6-producing cells in adipose tissue have not been identified. If IL-6 production is constitutive, as seems likely, the high levels associated with obesity may simply be due to the greater adipose tissue mass; a systemic response (low-grade APR) could be a predictable consequence of gaining weight, in particular enlarging one's abdominal girth. There also seem to be genetic influences (see below).

- IL-6-induced acute phase responses include many known CAD risk factors (see above):
  - Elevated blood fibrinogen, tPA
  - Low HDL-cholesterol, high VLDL
  - Decreased insulin sensitivity (TNF-α seems more important for this response)
  - Low serum albumin
  - High cortisol
  - High CRP, SAA
- A polymorphism (SNP) in the IL-6 promoter has been associated with LPS- or TNF-induced transcription of the IL-6 gene in vitro and with blood IL-6 levels in vivo (131). Fernandez-Real et al. found that subjects who were homozygous for the C allele at position –174 of the IL-6 gene had significantly lower fasting insulin levels and better performance on an oral GTT than did individuals who were carriers of the G allele (132). The same group reported that G carriers had significantly higher VLDL, higher free fatty acid levels, and lower HDL-cholesterol than did C homozygotes; a tendency toward higher IL-6 levels in the G carriers was also found (133) (see also (131)).
- Yudkin's analysis of 106 non-diabetic adults found a strong correlation ($r = 0.59$, $p < 0.00005$) between acute phase response molecules and insulin resistance syndrome variables (118).



**Plasma IL-6 levels according to genotype. Fishman 1999 J.Clin.Invest. 102:1369**

- In atherosclerosis-prone mice, administering IL-6 can accelerate the rate at which atherosclerotic changes develop (134).

Still missing is a convincing demonstration that selectively blocking IL-6 production or action can reduce the changes of the APR and prevent CAD. An important problem with such a study, as with many studies of inflammation-related cytokines, is that the target molecule (IL-6) plays very important roles in the body's acute responses to numerous stresses, including infection. Agents that potently interfere with IL-6 action may blunt these responses when they are most needed. On the other hand, there is evidence that both ASA and statins can prevent inflammation without impeding host defenses; they probably do this in part by blocking IL-6 synthesis. It should be possible to produce selective IL-6 antagonists with similarly low potency.

**Concluding comments**

The available evidence suggests strongly that CRP is sensitive, dynamic marker for the acute phase response. Whether it contributes directly to atherothrombosis is uncertain, although the epidemiological data are suggestive. (In some of the epidemiological studies, it remained as an independent predictor of CAD risk when numerous other variables have been included in multivariate analysis.) In any case, it is only one of many components of the APR – which, taken together, generates most of the known risk factors for CAD. Learning how to prevent low-level activation of the APR without interfering with its beneficial actions could reap great rewards for science and medicine.

It's important to note that the APR has other clinical consequences. Some are so common that we tend to minimize their frequency and importance.

1. **Hypoalbuminemia of acute illness.** At the onset of the APR, albumin synthesis abruptly ceases. Since the plasma half-life of albumin is 10 - 12 days, a decline in the serum albumin concentration occurs quickly. When severe, acute phase hypoalbuminemia can contribute to edema formation (even anasarca).
2. **Anemia of chronic inflammation** ("anemia of chronic disease")(135).
3. **Euthyroid sick syndrome.** The inflammation-induced decrease in $T_3$ is at least partly due to IL-6 (136), which (like TNF-α and IL-1) can decrease 5'-monodiodinase activity.
4. **Endogenous immunosuppression** Transient immunosuppression often follows severe trauma. The characteristics of this state suggest that it is caused by an exaggerated acute phase response – that the systemic anti-inflammatory forces become sufficiently strong to suppress innate host defenses at local sites of microbial invasion.

It's important to remember that the various components of the APR are not so highly orchestrated that they play the same tune in response to every kind of stimulus or in all individuals. There are both inherited and stimulus-specific differences. In some circumstances, CRP is not a useful way to monitor the APR – perhaps the best example for internists is systemic lupus, in which the ESR (fibrinogen) is much more responsive to disease activity than is CRP.

Finally, Yudkin et al. found that, among the residents of a single area in India, those living in rural communities had significantly lower blood levels of several APPs (and pro-inflammatory cytokines) than did those living in the city (either in slums or private dwellings)(Table at right)(137). Chronic, low-level activation of the APR may be a price that humans have paid for urbanization and the evolution of modern lifestyles.

| | Rural village | Urban slum | Urban middle class |
|---|---|---|---|
| N | 43 | 28 | 40 |
| Age | 28 | 35 | 38 |
| BMI ($kg/m^2$) | 18.7 | 22.3 | 23.3 |
| IL-6 (pg/ml) | 2.5 | 23.5 | 7.1 |
| TNF-α (pg/ml) | 2.6 | 39.3 | 31 |
| Leptin (pg/ml) | 1.9 | 9.6 | 6.3 |

**Modified slightly from Yudkin, et al. 1999. Diabetes Care 22:363**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