Brief Definitive Report

THE ACUTE-PHASE RESPONSE IN (NZB × NZW)F_1 AND MRL/l MICE

Contrasting Patterns Resembling those in Human Systemic Lupus Erythematosus and Rheumatoid Arthritis, Respectively*

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The acute-phase response of the plasma proteins to tissue injury and inflammation is a nonspecific phenomenon (1, 2). However, recent clinical work in man, using precise immunoassays, has shown that not all acute-phase proteins behave in a completely nonspecific way. For example, there are highly significant differences in the levels of C-reactive protein (CRP), the classical acute-phase reactant, and of serum amyloid A protein (SAA), the precursor of AA type amyloid fibrils, in different diseases (3, 4). Patients with active rheumatoid arthritis usually have high SAA (4) and CRP (5) levels, which correlate closely with other clinical and laboratory indices of disease activity (6, 7). In contrast, patients with systemic lupus erythematosus (SLE) generally fail to mount a major acute-phase response of CRP (8) or SAA (4), despite the presence of severe active disease. These patients, however, remain capable of producing CRP and SAA, and do so, for example, when they acquire intercurrent microbial infections (8).

Two distinct mechanisms may underlie such notable differences in acute-phase response. First, the disease process of SLE per se may not stimulate production of these particular acute-phase proteins. Second, those individuals who manifest SLE may be "low acute-phase responders," possibly on a genetic basis, to immune complex and autoantibody-mediated inflammation. The first explanation seems unlikely, as inflammation and tissue destruction on the scale seen in active SLE are usually powerful stimuli to acute-phase protein synthesis (1, 2), as are the active or passive formation of immune complexes in vivo (9, 10).

To investigate these questions further, we have studied the acute-phase response of serum amyloid P component (SAP) in two mouse models of spontaneous autoimmune disease. Mouse CRP is an acute-phase reactant, but is present only in trace amounts and is not easily measured; SAP, however, is closely related to CRP and is a major acute-phase protein in mice (11, 12). We report here for the first time that (NZB × NZW)F_1 (NZB × W) mice, which develop a chronic inflammatory disease resembling

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human SLE (13), completely fail to mount any acute-phase response of SAP during evolution of their pathology. In contrast MRL *lpr/lpr* (MRL/l) mice, which develop a more rapidly progressive lupus-like autoimmune disease including polyarthritis and the presence of serum rheumatoid factors (14, 15), display high levels of SAP correlating with the progress and severity of their disease.

Materials and Methods

Mice. BALB/c and MRL/l mice (The Jackson Laboratories, Bar Harbor, ME) were bred at the CIBA-GEIGY animal research center, Sisseln, Switzerland. These mice and female NZB × W animals (Bomholtgard, Denmark) were housed in the same room and fed the same diet from the age of 4 wk.

Blood samples. Groups of 5–16 mice were sequentially bled at the intervals shown in the figures, and sera were stored at −20°C before being tested individually at the end of the experiment.

Assays. The concentration of SAP in serum was measured by electroimmunoassay calibrated with the isolated pure protein (16). Antibodies to single stranded DNA (ssDNA) and to double stranded DNA (dsDNA) were measured separately by enzyme-linked immunoabsorbent assay (17), using as reference a serum pool from old female NZB × W mice, (arbitrary value 200 U/ml). Circulating immune complexes (CIC) were sought by solid-phase C1q binding assay (18), using an alkaline phosphatase-labeled rabbit anti-mouse IgG (specific for heavy and light chains) (N. L. Cappel Laboratories, Cochranville, PA) and standards of heat-aggregated mouse IgG. Proteinuria was assessed on random samples of urine using Rapignost sticks (Behringwerke AG, Marburg, Federal Republic of Germany) calibrated with mouse serum albumin (N. L. Cappel Laboratories).

Acute-phase Responses. Groups of five NZB × W and BALB/c mice at 1, 3, 6, and 9 mo of age received per mouse either 100 μg lipopolysaccharide (LPS) W from *Salmonella enteritidis* (Difco Laboratories, Detroit, MI) by intraperitoneal injection or 0.5 ml of 10% wt/vol casein solution (vitamin-free casein, ICN Nutritional Biochemicals, Cleveland, OH) by subcutaneous injection. Each mouse was bled before and 24, 48, and 72 h after the injection to provide serum for assay of SAP.

Results

With increasing age the concentrations of antibodies to ssDNA and dsDNA as well as the level of CIC increased in both NZB × W and MRL/l mice (Fig. 1). These serological changes correlated with the appearance and progression of clinical disease, exemplified by proteinuria, and the tempo of their progress was more rapid in the MRL/l mice, so that none survived to 40 wk. In the NZB × W mice there was a notable absence of any acute-phase response of SAP, whereas, in contrast, the MRL/l mice showed a dramatic rise in SAP levels, increasing from 50 ± 10 μg/ml at 6 wk to 420 ± 96 μg/ml at 25 wk (Fig. 1). Normal, untreated BALB/c mice, which served as controls, did not develop serological abnormalities and showed no acute-phase response (Fig. 1).

The capacity of NZB × W mice at any age between 1 and 9 mo to mount acute-phase responses to inflammatory stimuli other than their underlying diseases was demonstrated after injection of LPS or casein (Fig. 2). In both cases, the behavior of SAP was similar to that seen in control BALB/c mice.

Discussion

The different patterns of acute-phase response seen in different human diseases provide the basis for some useful applications of acute-phase protein measurement in clinical practice, but their underlying mechanisms are not known (2–5). The present

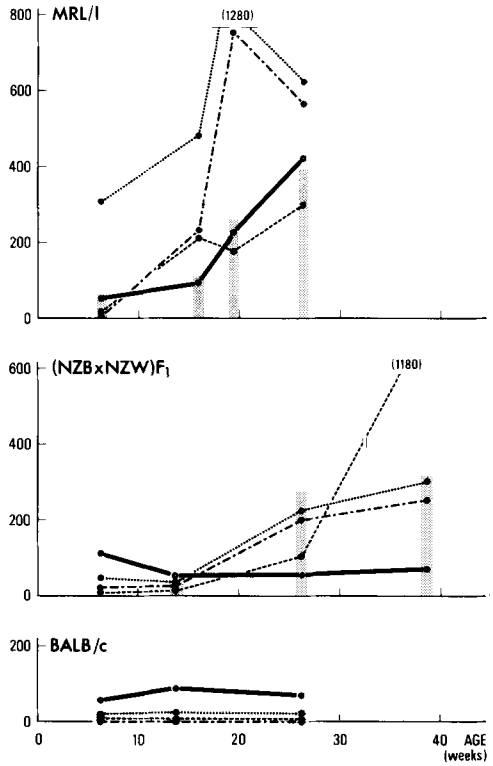

FIG. 1. Serum levels of SAP (——), CIC (– – –), anti-ssDNA antibodies (–·–), anti-dsDNA antibodies (·····), and quantity of proteinuria ( ) in autoimmune and control mice. Each point represents the arithmetic mean of 16 female (NZB × NZW)F_1, 6 male MRL/l, and 10 female BALB/c mice, respectively. Scale denotes 10^{-1} μg/ml for CIC, μg/ml for SAP, mg/dl for proteinuria, and U/ml of anti-DNA antibodies.

observations of different acute-phase responses of SAP, a protein closely related to CRP, in different mouse strains that spontaneously develop autoimmune, lupus-like, and rheumatoid arthritis-like diseases may help to elucidate these phenomena and also the control mechanisms of the acute-phase response in general.

CRP, SAP, and SAA are all synthesized by hepatocytes (19), and there is evidence that interleukin 1, derived from mononuclear phagocytes, may play an important role in the induction of their increased production (reviewed in 19). Prostaglandins may also be involved, as they can stimulate acute-phase responses both in the rabbit and man (20). The murine strains investigated here provide the opportunity to dissect the processes that underlie such differences in the acute-phase response in terms of production of mediators, such as interleukin 1 and prostaglandins, and responsiveness to different stimuli by macrophages and hepatocytes. It is intriguing that injection of prostaglandin E_1 has a significant protective effect both in NZB × W and MRL/l mice (21–23).

CRP and SAP are both ligand-binding proteins. CRP can react with a wide range of materials of exogenous and autogenous origin, including phospholipids, nucleic acids, and cationic intracellular proteins (reviewed in 2, 3), whereas SAP is able to bind fibronectin and C4-binding protein (24). SAA is an apolipoprotein of high

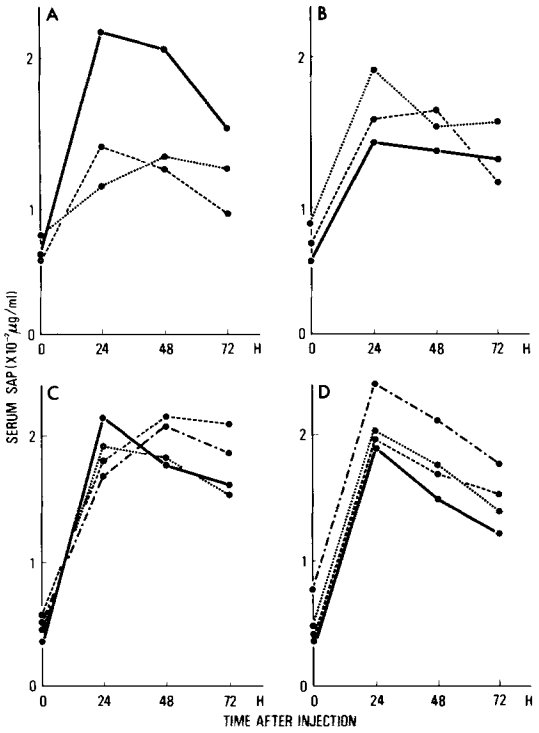

FIG. 2. Acute-phase responses of SAP to injection of LPS (A, C) or casein (B, D) in (NZB × NZW)F_1 (C, D) and BALB/c mice (A, B). Each point represents the arithmetic mean of five female mice, groups of which were tested at the age of 1 (——), 3 (• • • • •), 6 (– – –), and 9 mo (–•–).

density lipoprotein (reviewed in 19), and this class of lipoprotein has recently been shown to form complexes with gram-negative bacterial endotoxin in the plasma (25). The rate of production and the circulating level of these proteins may thus influence the outcome of infective or noninfective inflammation or tissue damaging events and modify clinical patterns of disease.

We have previously reported (11) that normal resting levels of SAP are genetically determined in mice. The present finding of failure of NZB × W mice to mount an acute-phase SAP response to progress of their disease closely resembles the situation with regard to CRP in human SLE, whereas the response of MRL/l mice, in contrast, parallels that seen in human rheumatoid arthritis or those exceptional individuals with SLE whose patterns of disease alters to become more like systemic vasculitis (5, 8). These analogies of response raise the possibility that production of human acute-phase proteins in response to some stimuli may also be under genetic control, and suggest that this could contribute towards individual susceptibility to particular autoimmune or other chronic inflammatory disease. Further exploration of these phenomena in the mouse models should therefore be both of academic interest and clinically relevant.

Summary

The acute-phase plasma protein response to disease activity in murine models of autoimmune lupus-like disease was investigated by measurement of the concentration

of serum amyloid P component (SAP) in NZB × W and MRL/l mice. The levels of SAP, which is a major acute-phase protein in mice, did not rise at all in response to progression of disease in NZB × W mice between the ages of 1 and 9 mo. This resembles the behavior of acute-phase proteins such as C-reactive protein and serum amyloid A protein in human systemic lupus erythematosus, and just as in human lupus, where the occurrence of intercurrent microbial infection can stimulate an acute-phase response, so injection of bacterial lipopolysaccharide or casein into the NZB × W mice stimulated "normal" acute-phase SAP production. In marked contrast, MRL/l mice developed greatly increased levels of SAP, which correlated closely with progression of their pathology as they aged. The disease profile of the MRL/l strain includes rheumatoid factors and spontaneous polyarthritis and their SAP response resembles the behavior of acute phase proteins in human rheumatoid arthritis. Different patterns of acute-phase response in different autoimmune disorders may thus be a reflection of the genetic predisposition to particular diseases and/or contribute to their pathogenesis. The existence of animal counterparts for the various clinical patterns of human acute-phase protein production will assist in experimental investigation of the underlying mechanisms and of the biological role of the acute-phase response.

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