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Complement gene expression in hepatic and extrahepatic tissues of NZB and NZB × W (F_1) mouse strains

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SUMMARY

To study the role of local production of complement proteins during the evolution of a naturally occurring immune complex disease, C3, C4, C2 and Factor B mRNA expression was assessed in several tissues of the inbred mouse strains NZB and (NZB × W) F_1 hybrid. In the NZB/W F_1 hybrid strain, coincident with the development of glomerulonephritis a marked increase in kidney C3 and C4 mRNA was observed; Factor B mRNA, which is expressed as a doublet in kidney and intestine, showed an increase in expression of the smaller transcript. This alteration of kidney C3, C4 and Factor B mRNA is identical to that noted in association with lupus nephritis in the MRL lpr/lpr strain and following *in vivo* administration of endotoxin to the BALB/c strain. The development of systemic lupus erythematosis (SLE) in the NZB/W F_1 was not associated with a marked change in hepatic complement gene expression. These findings support the hypothesis that local production of complement may play a role in the pathogenesis of glomerulonephritis and other tissue injury in SLE.

INTRODUCTION

Immunohistological studies in patients with systemic lupus erythematosis (SLE) or in the naturally occurring murine model of SLE show increasing accumulation of C3 with progression of the disease, particularly in the kidney, but also in other affected organs. Complement is thought to be important in the pathogenesis of the tissue injury accompanying SLE (Couser, 1985). Tissue deposition of complement is clearly implicated pathophysiologically in some forms of experimental membranous nephritis, since prior depletion of C3 by treatment with cobra venom factor abrogates the disease (Salant *et al.*, 1980). In the chronic immune complex disease of SLE, decreased serum concentrations of complement correlate with clinical exacerbations (Kimberly *et al.*, 1983; Atkinson, 1986). These decreased concentrations of C3 are due to increased utilization and decreased hepatic synthesis (Alper & Rosen, 1967).

Immune deposits in tissues may arise by local formation or by deposition of immune complexes. In lupus nephritis these complexes are characteristically located within the mesangial region of the glomerulus (Atkinson, 1968). In several experimental models, including the (NZB × W) F_1 mouse strain, macrophages have been shown to accumulate in the glomerular mesangial region with progression of nephritis (Schreiner, Cotran & Unanue, 1984; Holdsworth, Neale & Wilson, 1981; Kimura *et al.*, 1987). We have also demonstrated, using a F4/80 monoclonal antibody that detects a mouse macrophage membrane-specific antigen, a marked increase in content of macrophages in the glomerulus and renal interstitium of the MRL lpr/lpr mouse strain with progression of glomerulonephritis (Passwell *et al.*, 1988). Both macrophages and mesangial cells may take up immune complexes and produce inflammatory mediators, including prostaglandin E_2, coagulant factors and various cytokines (Neuwirth *et al.*, 1988; Pinckard, Ludwig & McManus, 1988; Dinarello, 1984).

Independent regulation of complement gene expression in hepatic and extrahepatic tissues has been documented in humans and experimental animals (Cole *et al.*, 1985; Falus *et al.*, 1987a). In a study of complement gene expression in a mouse strain (MRL lpr/lpr) that exhibits a rapidly progressive murine SLE, we demonstrated an increase in Factor B, C3, C4 and C2 mRNA and an increase in synthesis of Factor B and C3 proteins in kidney in association with the development of glomerulonephritis (Passwell *et al.*, 1988). Other target organs manifesting autoimmune arteritis, such as lung and heart, also displayed increases in C3, C4 and C2 and Factor B mRNA in comparison to the corresponding tissues of the control, congenic MRL +/+ strain. For some (e.g. C3) the increase in mRNA at extrahepatic sites with advancing disease exceeded the changes in the corresponding liver expression of specific mRNA at each time-point examined. These findings indicate that local regulation of these genes in naturally evolving disease may be important in the immunopathogenesis of some autoimmune diseases.

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The purpose of this study was to determine changes in expression of the C3, C4, C2 and Factor B genes in the kidneys of the NZB and NZB × W (F_1) mouse strains during the development of chronic glomerulonephritis to assess local complement production in target organs of another murine SLE model, and thus to test the generality of our hypothesis that local, tissue-specific production of complement plays a pathogenetic role in SLE.

MATERIALS AND METHODS

Mice

Female mice of the NZB and NZB × W (F_1) hybrid strain were purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained on a regular diet. Urine was collected from groups of mice in metabolic cages and proteinuria was quantified by sulphosalicyclic acid precipitation. At ages 2, 4, 6 and 8 months, mice of each strain were killed by cervical dislocation, bled by intracardiac puncture, and kidneys, livers, spleen, lungs, intestines and heart rapidly removed. As the NZB strain survives longer, an additional point at 10 months was included; the kidneys of 10-month-old BALB/c mice were used as an additional control. All organs were frozen in liquid nitrogen and stored at −70° until processed for isolation of total RNA. EDTA plasma was also stored at −70° until analysed for C3 and creatinine concentrations. C3 concentration in the plasma was measured by immunodiffusion using a rabbit anti-mouse C3 antibody, kindly provided by Dr Sakai (Kanazawa, Japan). Eight-week-old BALB/c mouse plasma (concentration 50 mg/dl) was used as standard. Progression of kidney disease was monitored by histological examination of the kidneys at various time intervals. Kidney sections were embedded in paraffin, stained with haematoxylin, eosin and PAS, and reviewed in a blind fashion. The severity of renal injury was classified by grading the histological sections for glomerulonephritis, glomerulosclerosis and interstitial inflammation. The details of the scoring scale were as described previously (Passwell *et al.*, 1988).

Probes

For C2 mRNA, the 800 bp Pst I fragment of the pC2M mouse cDNA clone was used (Falus *et al.*, 1987b). The C4 probe was the 1·4 kb fragment of the clone $pFc_4/5{\cdot}4$ (Nonaka *et al.*, 1985). Chicken α-actin cDNA (Schwartz *et al.*, 1980), which also hybridizes to beta and gamma actin, was used. RNA probes for Factor B and C3 were prepared from their respective DNA clones and subclones into pGEM, as described previously (Falus *et al.*, 1987a). The transcribed single-stranded RNA was labelled with ^{32}P-UTP (Amersham, Arlington, IL) according to the manufacturer's protocol (Promega, Madison, WI).

RNA isolation and Northern blot analysis

The methods used for mRNA isolation and Northern blotting have been described in detail elsewhere (Passwell *et al.*, 1988). Briefly, frozen tissues were pulverized in liquid nitrogen and lysed in 4 M guanidine thiocyanate, then subjected to sequential centrifugation through cesium chloride and ethanol precipitation. The resulting RNA content was estimated by absorbance at 260 nm.

RNA samples were then size separated by 1% agarose formaldehyde gel electrophoresis, transferred to nylon membranes and fixation was accomplished by UV light exposure.

Prehybridization and hybridization with specific RNA probes were performed at 55–60° in 50% formamide, 50 mM sodium phosphate, pH 6·5, 0·8% NaCl, 1 mM EDTA, 0·5% Denhardt's solution, 500 μg/ml denatured salmon sperm DNA and 500 μg/ml yeast RNA (Sigma Company, St Louis, MO). Filters were washed at 65° in 50 mM NaCl, 20 mM sodium phosphate, pH 6·5, 1 mM EDTA and 0·1% SDS. Semi-quantitative analysis of the amount of increase in mRNA was estimated either by laser densitometry of the autoradiographs and/or by cutting the blots and determining the radioactivity in specific bands by scintillation counting.

RESULTS

Clinical findings and histology

At 8 months of age, marked proteinuria was noted in the F_1 hybrid strain, in association with an increase in creatinine concentration. Only slight to moderate proteinuria and stable creatinine levels were observed in the NZB strain. The plasma C3 levels were not markedly altered in the F_1 hybrids during the time they were studied; the NZB strain did show lower C3 concentrations at 2 months of age (Table 1).

No lesions were observed in either NZB or NZB/W F_1 strains until 11 weeks. At that time, the kidney of NZB mice

Table 1. Renal histology and clinical parameters in NZB and NZB/W F_1 strains

	NZB					NAB/W F_1			
Age (weeks)	6	11	18	32	40	6	11	18	32
Glomerulonephritis	−	+	++	++	++	−	++	++	+++
Glomerular sclerosis	−	−	−	−	−	−	−	−	+++
Interstitial nephritis	−	−	−	−	−	−	−	−	+++
Urine protein* (mg/ml)	1·05			0·2		ND			19·43
Plasma creatinine* (mg/ml)	0·1			0·2		0·2			0·5
Plasma C3† (mg/ml)	39±4			62±7		52±8			50±13

* Average of two to three mice at each time.

† Mean ± SEM three to five mice at each time.

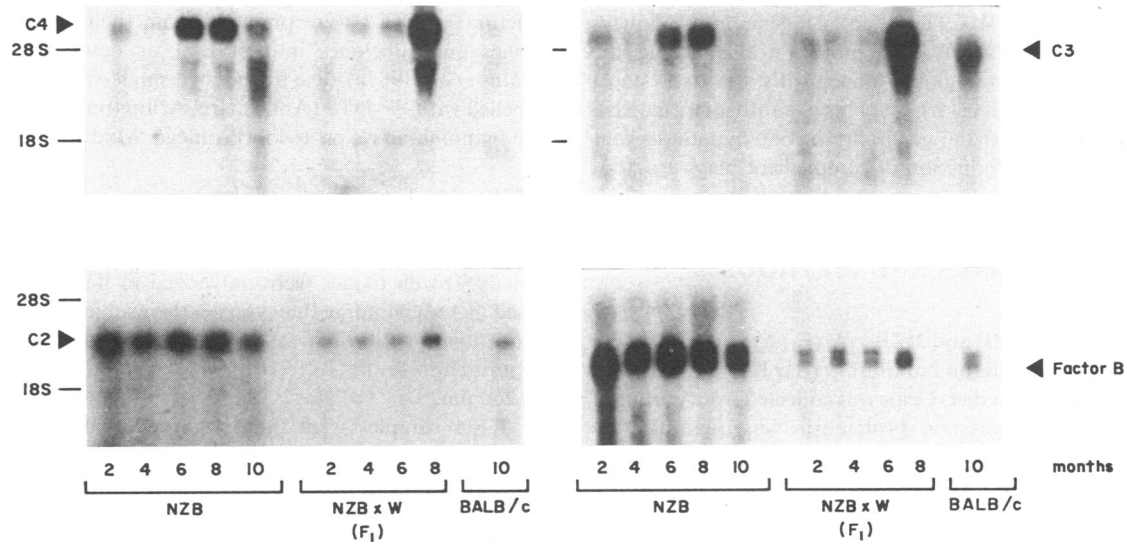

Figure 1. Northern blot analysis of C4, C3, C2 and Factor B mRNA in kidney of NZB and NZB/W (F_1) mouse strains at timed intervals. 10 μg of total RNA were loaded in each lane. Similar results were recorded from four animals at 2, 4 and 8 months and two animals at age 6 months. There was no significant difference in amount of RNA loaded in each, as estimated by ethidium bromide stain and actin mRNA content.

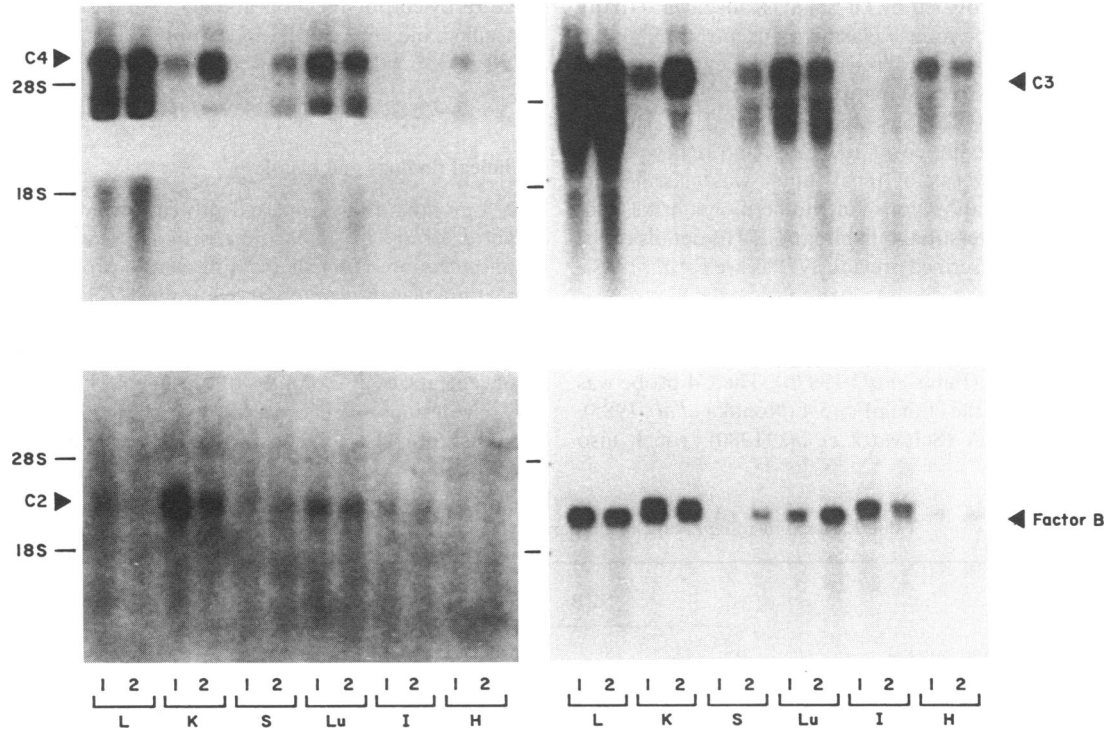

Figure 2. Comparison between NZB and NZB/W (F_1) expression of C4, C3, C2 and Factor B mRNA in various organs of mice, age 8 months. 10 μg RNA were loaded in each lane (except lane 1 and 2 for liver, 5 μg/lane). L, liver; K, kidney, S, spleen; Lu, lung; I, intestine; H, heart. First sample of each pair from NZB and second from NZB × W (F_1). This experiment is one of three separate Northern blots performed on each of two to three individual mice. The C4 autoradiograph was performed by hybridizing with the C4- specific probe after the Factor B bands were cut out for quantification of specific radioactivity. No C4 mRNA species with a mobility faster than the 28S marker have been observed.

displayed a mild, focal mesangial hypercellularity, while NZB/W F_1 mice manifested a diffuse mesangioproliferation with expansion of mesangial matrix and hypercellularity. The NZB strain evolved a similar lesion by 18 weeks, which had progressed no further by 40 weeks. Throughout the course of the experiment, no interstitial infiltrate, interstitial fibrosis or glomerular sclerosis was observed. In contrast, the NZB/W F_1 strain progressed to a diffuse, proliferative glomerulonephritis at 32 weeks. This was accompanied by an interstitial infiltrate, perivascular accumulation of leucocytes, focal tubular dilatation and atrophy, and patchy interstitial fibrosis. Widespread, segmental glomerular sclerosis was observed.

mRNA expression of C2, C4, C3 and Factor B in organs of NZB and NZB/W F_1 mice

We chose age 8 months as the critical time-point to compare the relative amounts of specific complement mRNA in kidneys of the two strains and also the relative amounts expressed in each organ as a function of SLE manifestations. Previous studies have shown that in the NZB/W strain autoimmune disease is well-established at this time-point. At 2 months C3 and C4 mRNA expression in the kidney of NZB were at the most twofold greater than in the NZB/W F_1, but C2 and the factor B short transcript in the NZB were more than four- to five fold greater than the F_1 kidney (Fig. 1). Striking increases in C3, C4, C2 and factor B short transcript expression, most marked in the NZB/W F_1, was observed by 8 months. C2 and Factor B expression in kidney of the NZB strain remained relatively constant (though higher than in the F_1) throughout the observation period.

In liver, C3 and Factor B expression at 2 months was approximately twofold more in the NZB compared to the F_1(2·2 versus 1·3 for C3 and 1·9 versus 1·1 for Factor B when assessed by densitometry and corrected for actin mRNA as an estimate of gel loading) (data not shown), but at 8 months no significant differences in liver expression of any of the complement genes (C2, C3, C4, Factor B) were recognized (e.g. for Factor B the actin corrected densitometry was 1·2 for NZB versus 1·4 for the F_1) (Fig. 2). Splenic C3, C4 and Factor B were significantly greater in the F_1 than in the NZB. Marked splenomegaly was noted in the F_1 hybrids but not quantified to avoid degradation of splenic mRNA. A well-described lymphoid hyperplasia has been recognized in the lungs of the NZB strain but not the F_1 strain; both stains had evidence of coronary arteritis (Theofilopoulos & Dixon, 1985). Associated with this pulmonary inflammation C3, C4 and, to a lesser extent, C2 mRNA were greater in the NZB than the F_1. However, Factor B mRNA in the F_1 lung was reproducibly greater than the corresponding mRNA in NZB mice. Likewise, cardiac C3 mRNA in the NZB was more than in heart of the F_1 animals.

DISCUSSION

In this study of complement expression in NZB and NZB × W (F_1) mice, increases in extrahepatic expression of mRNA for C3, C4 and Factor B, with less marked change of C2 mRNA, were noted in association with advancing signs of inflammation in target organs. In contrast, mRNA concentrations of the corresponding complement genes in the liver did not vary with progression of disease. Differences in hepatic mRNA expression between the strains in the juvenile animals were relatively small. In the NZB/W kidney, a marked increase in C3 and C4 expression at 8 months of age coincided with the development of a florid glomerulonephritis. We have noted similar increases in C3, C4, C2 and Factor B kidney mRNA expression in the MRL lpr/lpr mouse strain in association with disease progression (Passwell *et al.*, 1988).

Two Factor B transcripts in kidney and intestine have now been identified in several mouse species.The long Factor B transcript, which is prominent in kidney and intestine but accounts for $< 10\%$ of Factor B transcripts in other organs, originates from an alternative upstream initiation site; the short Factor B transcript is the major transcript (initiated downstream) in isolated hepatocyte and macrophage cell cultures (Nonaka, Gitlin & Colten, 1989a; Nonaka *et al.*, 1989b). In the NZB/W kidney the increase in Factor B expression was entirely accounted for by an increase in the Factor B short transcript. An identical change was observed in the kidneys of MRL lpr/lpr mice with progression of glomerulonephritis and in BALB/c mice following endotoxin administration *in vivo* (Falus *et al.*, 1987a). Thus, in the present and previous studies, evidence has been obtained for independent regulation of the two Factor B transcripts.

The NZB mouse, which served as control, had a survival time of approximately 430 days. Morphological evidence of mesangioproliferative glomerulonephritis, present as early as 11 weeks, was followed by gradual progression of renal disease, but did not become as florid as in the F_1 hybrid strain. By 10 months of age glomerular sclerosis was evident only in the F_1 (NZB/W) strain. NZB mice are C5 deficient and it is possible that this serves as a protective mechanism limiting complement-dependent immunopathology (Theofilopoulos & Dixon, 1985). Indeed, in a recent study comparing C5-sufficient and -deficient mice, a marked decrease in severity and incidence of an experimentally induced glomerulonephritis was observed in the C5- deficient mice (Falk & Jennette, 1986). Constitutive expression of Factor B and C2 mRNA in the kidney of young NZB animals exceeds that in the F_1 strain, so that at least for Factor B and C2 differences between the strains in mRNA concentration cannot be directly correlated with severity of disease. Similar differences in hepatic and extrahepatic constitutive expression of complement genes have been noted in other murine inbred strains (Falus *et al.*, 1987a).

This and previous studies provide evidence for substantial increases in complement production at local sites of inflammation. Many different cell types, including epithelia, fibroblasts, mononuclear phagocytes, adipocytes, endothelia, etc., have been shown to synthesize and secrete complement proteins (Colten & Gitlin, 1989). The regulation of complement gene expression is mediated by similar, but not identical, mechanisms among these various cell types. Increased local production of inflammatory cytokines (tumour necrosis factor, interleukin-1), which do increase in association with development of murine SLE, (Boswell *et al.*, 1988a, b), and others which exacerbate the disease (interferon-gamma; Adam *et al.*, 1980) could up-regulate complement gene expression in the fixed tissue cells of the individual SLE target organs. However, the inflammatory exudate itself contains complement- producing cells so that the net increase in complement expression may be a product of both sources. In either event, data from this study and that of complement gene expression in MRL mice indicate that interpretations of the role of complement pathogenetically must be

derived from estimates of net local complement production and not from changes in serum complement alone.

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