Regulation of Class III Major Histocompatibility Complex Gene Products by Interleukin-1

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Interleukin-1 (IL-1) is a product of mononuclear phagocytes that mediates changes characteristic of the response to inflammation or tissue injury (the acute-phase response). One of two structurally and functionally homologous major histocompatibility complex (MHC) class III genes encodes a positive acute-phase protein, complement factor B. The closely linked complement C2 gene is not affected during the acute-phase response. Purified human IL-1, *p*H 7.0, and recombinant-generated murine IL-1, *p*H 5.0, increased the expression of factor B and other positive acute-phase proteins in human hepatoma cells but decreased the expression of albumin, a negative acute-phase reactant. Furthermore, in a murine fibroblast L-cell line transfected with cosmid DNA bearing the human C2 and factor B genes, IL-1 mediated a reversible dose- and time-dependent increase in factor B expression in the transfected cells. Expression of the C2 gene was not affected by IL-1. The effect of IL-1 on factor B expression involves a mechanism acting at a pre-translational level as demonstrated by an increase in specific messenger RNA content and a corresponding increase in biosynthesis and secretion of factor B. The structural basis and mechanism for selective and independent regulation of these genes provides insight into the molecular control of the inflammatory response.

DURING THE SYSTEMIC REACTION to inflammation or tissue injury—the acute-phase response—the plasma concentrations of several liver-derived glycoproteins increase while those of others decrease. Addition of a single mediator, interleukin-1 (IL-1), to murine hepatocytes in primary culture can effect many, if not all, of the positive and negative changes in specific gene expression characteristic of the acute-phase response (*1*). To define the molecular basis and tissue specificity of IL-1–dependent modulation of acute-phase gene expression, we used as models human hepatoma Hep3B cells and murine fibroblast L cells transfected with DNA bearing genes that encode a positive acute-phase reactant, factor B, and the homologous protein, the second component of complement (C2).

Human hepatoma-derived Hep3B cells were incubated with purified human IL-1 and then with a radiolabeled amino acid precursor. Biosynthesis and secretion of specific proteins were analyzed by immunoprecipitation, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and fluorography. IL-1 mediated an increase in synthesis and secretion of complement proteins factor B and C3 but had no effect on synthesis and secretion of C2 (Fig. 1A). IL-1 also mediated a dose-dependent decrease in synthesis and secretion of albumin (Fig. 1B).

Close linkage of the C2 and factor B genes

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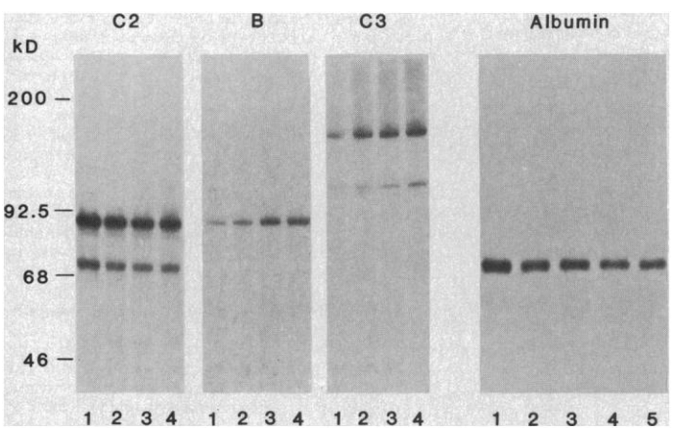

Fig. 1 (left). Effect of IL-1 on (A) C2, factor B, and C3 and (B) albumin synthesis by human hepatoma cells. Hep3B cells were maintained in culture as previously described (*10*). Confluent monolayers were incubated (A) without IL-1 (lane 1) or with IL-1 at (lane 2) 0.4 unit/ml, (lane 3) 2.0 unit/ml, (lane 4) 10.0 unit/ml or (B) with IL-1 at (lane 2) 0.1 unit/ml, (lane 3) 0.5 unit/ml, (lane 4) 2.5 unit/ml, and (lane 5) 12.5 unit/ml for 18 hours. Monolayers were then rinsed several times prior to 20-minute incubation in L-[^{35}S]methionine and Dulbecco's modified essential medium lacking methionine (250 μCi/ml). Cell culture fluid was then harvested, and cells were solubilized in 1% Triton X-100, 0.5% deoxycholic acid, 10 m*M* EDTA, 2 m*M* phenylmethylsulfonyl fluoride, and leupeptin and pepstatin at 100 μg/ml each in phosphate-buffered saline, *p*H 7.6, and then were subjected to two freeze-thaw cycles. Cell culture fluid and lysates were clarified by centrifugation at 15,000*g* for 30 minutes and stored at −70°C. Aliquots of the cell lysates were precipitated with the appropriate antisera and staphylococci-bearing protein A for 7.5% SDS-PAGE and fluorography as previously described (*11*). IL-1 did not affect secretion of C2, factor B, C3, or albumin, since similar relative changes were demonstrated in cell lysates and cell culture fluid. Cell number and total protein synthesis, as determined by trichloroacetic acid precipitation (*12*), were independent of IL-1 input. Molecular mass markers are indicated.

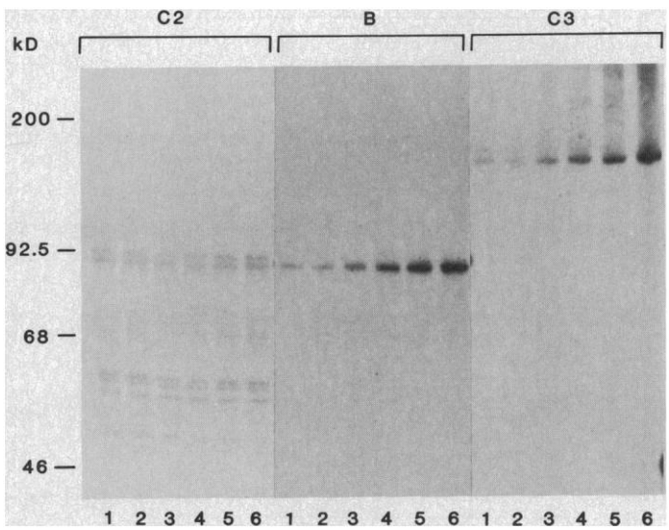

Fig. 2 (right). Effect of IL-1 on C2, factor B, and C3 biosynthesis by L cells transfected with human DNA bearing the C2 and factor B genes. Confluent monolayers of transfected L cells were incubated without IL-1 (lanes 1 and 2) or with human IL-1 at (lane 3) 0.4 unit/ml, (lane 4) 2.0 unit/ml, (lane 5) 10.0 unit/ml, and (lane 6) 40.0 unit/ml for 18 hours. Transfected L cells were allowed to incorporate radiolabeled methionine, and newly synthesized, radiolabeled C2, factor B, and C3 in the cell lysates were detected as described in Fig. 1. IL-1 did not affect the secretion of these proteins. Total protein synthesis was 22.8% higher in lanes 5 and 6 than in lanes 1 to 4 in this experiment. In other experiments, however, total protein synthesis was not affected by IL-1. Molecular mass markers are indicated.

within the class III region of the MHC [the 3′ end of the C2 gene is within ~450 base pairs of the 5′ end of the factor B gene (*2*)] permitted isolation of a cosmid DNA fragment bearing both genes. Stable cell lines, mouse L-cell lines transfected with this cosmid DNA fragment, synthesize and secrete C2 and factor B (*3*). These transfected L cells were incubated without IL-1 (Fig. 2, lanes 1 and 2) or with increasing concentrations of human IL-1 (lanes 3 to 6). There was a dose-dependent increase in synthesis of factor B and a dose-dependent increase in the synthesis of the endogenous, constitutively expressed C3 gene (Fig. 2). Factor B was not detected in L cells transfected with a control plasmid, even after incubation in medium containing the highest concentrations of IL-1. Expression of the C2 gene was not affected by IL-1 (Fig. 2). It might be argued that the lack of effect of IL-1 on C2 in transfected L cells resulted from transfection of cosmid DNA lacking the regulatory regions of the C2 gene. Several experiments were conducted to test this possibility. First, the C2 gene was constitutively expressed, suggesting that sequences required for transcription of the gene were transferred into the L cells. Second, IL-1 had no effect on the expression of C2 in murine hepatocytes (*1*) or human hepatoma cells, or on the expression of endogenous murine L-cell C2. Third, regulatory sequences necessary for enhanced expression of C2 by interferon-γ were also present within the transferred human DNA (*4*). The possibility that the lack of effect of IL-1 on C2 in L cells resulted from rearrangement of the C2 and factor B genes during transfection was also considered. Southern blot analysis of transfected L-cell genomic DNA with radiolabeled C2 and factor B complementary DNA (cDNA) probes revealed co-migrating 8.4-kb Pvu II and 2.7-kb Pvu II + Hind III fragments (Fig. 3). These fragments were also identified in human leukocyte DNA as previously described (*2*), but not in L cells transfected with the control plasmid. Finally, the increase in factor B expression without change in C2 expression was observed in two L-cell lines independently transfected with cosmid DNA bearing both genes.

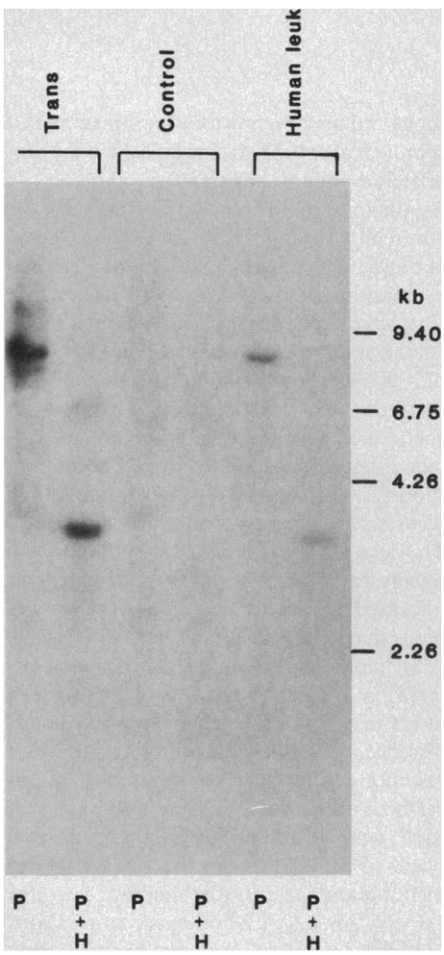

Fig. 3. Southern blot analysis of transfected L-cell genomic DNA. High molecular weight DNA was isolated by previously described techniques (*13*). Ten micrograms of DNA from L cells transfected with the C2 and factor B genes (Trans), L cells transfected with control plasmid (Control), and human leukocytes (Human leuk) was digested to completion with Pvu II (P) or Pvu II + Hind III (P + H) and fractionated by 0.7% agarose gel electrophoresis prior to Southern blot transfer. The blot was hybridized with a ^{32}P-labeled cDNA probe for human factor B (*14*), and fragments were identified by autoradiography. The 8.4-kb Pvu II and 2.7-kb Pvu II + Hind III fragments of transfected L-cell and human leukocyte DNA were also identified in autoradiography after hybridization with ^{32}P-labeled cDNA probe for C2. DNA size markers are indicated.

The effect of IL-1 on factor B and C3 expression involved a mechanism acting at a pre-translational level. After incubation with IL-1, total cellular factor B mRNA concentration increased (Fig. 4), while C2 mRNA was not affected. Murine C3 mRNA concentration also increased after incubation with IL-1. The increase in factor B expression due to IL-1 was reversible and time-dependent. The rate of synthesis of factor B increased by 2 hours and continued to increase at 15 hours of incubation in IL-1-containing medium. The effect was reversed within 2 and 6 hours after removal of IL-1 from the cell culture fluid.

The effect of IL-1 on factor B expression in transfected L cells required fivefold higher concentrations [based on bioactivity (*5*)] of recombinant-generated murine IL-1 (*6*) than of IL-1 purified from the cell culture fluid of human peripheral blood monocytes (*1, 7*). Similar relative ratios were required for the effect on endogenous murine C3 expression by transfected L cells. This difference in dose response has also been observed in Hep3B cells and isolated murine hepatocytes (*1*). This difference could result from differences in method of isolation or from primary structural differences between the murine and human IL-1 polypeptides (*5, 8*). Nevertheless, the fact that both human and murine IL-1 produce the same response indicates that the response is species-independent.

These experiments demonstrate that IL-1 has different effects on the expression of two genes that share many structural and functional characteristics and that appear to have evolved from a common ancestral gene (*2, 3, 9*). This differential effect of IL-1 is probably due to differences in the C2 and factor B genes themselves, since the response in human hepatoma cells was reproduced in murine fibroblasts after DNA-mediated gene transfer. Furthermore, the small size of the intergene segment (5′ flanking region of the factor B gene), limited by the 3′ terminus of the IL-1-unresponsive C2 gene, provides a well-defined region to probe the structural basis for differential regulation of these two

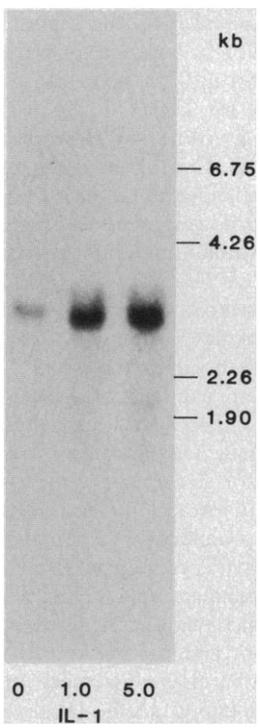

Fig. 4. RNA blot analysis of the effect of IL-1 on factor B expression by L cells transfected with human DNA bearing the C2 and factor B genes. Total cellular RNA was isolated in guanidine isothiocyanate (*15*) after an 18-hour incubation of transfected L cells in medium without IL-1 or with IL-1 at concentrations of 1.0 and 5.0 unit/ml. RNA was denatured by heating in formaldehyde and fractionated in agarose-formaldehyde electrophoresis prior to nitrocellulose transfer (*16*). RNA blots were then hybridized with radiolabeled cDNA probes for human factor B (*14*), human C2 (*17*), and murine C3 (a gift of M. Takahashi, Kanazawa, Japan) prior to autoradiography.

closely related genes and the regulation of other genes modulated during acute inflammation. Finally, these results indicate that a single mediator, IL-1, can induce increases and decreases in the expression of genes encoding liver-derived plasma proteins that are affected during the acute-phase response.

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Lower Cretaceous Angiosperm Flowers: Fossil Evidence on Early Radiation of Dicotyledons

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Three-dimensionally preserved unisexual angiosperm flowers and inflorescences have been recovered from the Lower Cretaceous Patapsco Formation (Potomac Group) of eastern North America, in sediments palynologically dated as late Albian, approximately 100 million years old. In situ tricolpate pollen shows that the flowers were produced by some of the earliest higher (nonmagnoliid) dicotyledons, and the morphology of pollen, flowers, and inflorescences indicates a close relation to extant Platanaceae. Combined with architectural and cuticular features of associated leaves these floral remains suggest that *Platanus*-like plants with unisexual, probably insect-pollinated flowers were an important element in the mid-Cretaceous diversification of dicotyledonous flowering plants.

PALEOBOTANICAL AND STRATIGRAPHIC analyses during the last 30 years have documented a major radiation of flowering plants during the mid-Cretaceous (*1–3*). Between the Hauterivian and Cenomanian, unequivocal angiosperm pollen and leaves first appear in the fossil record and exhibit parallel patterns of increasing diversity, complexity, and abundance (*1–5*). Available evidence suggests that this reflects a major systematic and ecological radiation, during which many of the features of pollen morphology and leaf architecture that characterize extant flowering plants appeared for the first time (*5, 6*). Although detailed studies of fossil leaves and pollen have begun to clarify the systematic relations of mid-Cretaceous angiosperms (*3, 5–9*), many critical aspects of their structure and biology remain inaccessible from studies of leaves and pollen alone. Where available, mid-Cretaceous flowers supply unequivocal evidence of systematically important floral structure and provide an improved basis for interpreting pollination and other aspects of early angiosperm biology (*10, 11*).

Extant dicotyledons are divided into six subclasses: Magnoliidae, Hamamelidae, Caryophyllidae, Dilleniidae, Rosidae, and Asteridae. The higher (nonmagnoliid) dicotyledons include over 70 percent of extant angiosperm species (*12*), and triaperturate pollen diagnostic of this group first appears at very low concentrations in the Barremian-Aptian of Northern Gondwana (*13*), approximately 120 million years ago. During the Aptian and Albian, the variety and abundance of triaperturate pollen increases dramatically, and by the middle Cenomanian, approximately 25 million years later, many palynofloras include tricolpate, tricolporoidate, tricolporate, and triporate forms (*1–3*). This clear chronological pattern in the fossil pollen record has been interpreted as reflecting the initial major radiation of nonmagnoliid dicotyledons.

The Early Cretaceous plants that produced tricolpate pollen have not been identified, but studies of foliar remains suggest that they may have been early representatives of the Hamamelidae and Rosidae, represented by so-called platanoid and *Sapindopsis* leaves, respectively (*2, 5, 9*). The fossil flowers discussed here provide information on floral structure in this important group of flowering plants during the Early Cretaceous and predate previous reports of angiosperm flowers with in situ pollen by approximately 6 million years (*10*).

Fossil inflorescences and flowers were recovered by sieving from a grey clay collected at the West Brothers locality in the Patapsco Formation (Potomac Group) of Maryland. The associated palynoflora suggests a late Albian age, approximately 100 million years old (*2, 14*). The fossiliferous sediments are lenticular and interpreted as the fill of an abandoned channel (*15*). They contain wood fragments, conifer cones, seeds and shoots, and a variety of angiosperm fruits, seeds, and other reproductive structures. The leaf flora is dominated by *Sapindopsis* foliage, and the diversity of angiosperm reproductive structures recovered far exceeds that of the associated fossil leaves.

Staminate and pistillate flowers are clustered in separate, more or less spherical heads that are sessile on an elongated inflorescence axis. Although both kinds of inflorescence occur separately, the similar spherical heads and floral structure suggest that they were derived from closely related plants. Pistillate flowers consist of several membranous tepals surrounding five free carpels (Fig. 1A). The outer tepals are short, but the inner are longer and frequently extend to the apex of the carpels. Clumps of pollen identical to that preserved in the staminate flowers occur at the apex of some of the inner tepals. Stamens are not present, but the possibility that some of the inner tepals may be staminodes cannot be resolved with present material. The carpels are oblong, with an incompletely fused adaxial suture extending for most of their length.

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