Blut (1985) 51: 267–274

Blut

Cytokines Regulating Acute Inflammation and Synthesis of Acute Phase Proteins

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Summary. The acute phase response to injury includes metabolic alterations, such as fever, leucocytosis, enhanced uptake of some metals and amino acids by liver, and changes in the synthesis of certain plasma proteins. Many of these effects can be elicited either in vivo or in tissue culture by monocyte- and keratinocyte-derived cytokine interleukin 1 (IL-1), which had earlier been variably termed leucocytic endogenous mediator, lymphocyte activating factor, or endogenous pyrogen. Although recombinant murine IL-1 was shown to induce hepatic synthesis of acute phase proteins other authors demonstrated that hepatocyte stimulating factor (HSF) is distinct from IL-1. Possible relationships between HSF und IL-1 and the molecular mechanisms of action of these cytokines on the synthesis of acute phase proteins are briefly discussed.

Key words: Cytokines – Interleukin 1 – Interleukin 2 – Lymphocyte activating factor – Endogenous pyrogen – Hepatocyte stimulating factor – Prostaglandins – Stimulated macrophages – Acute phase proteins – Fibrinogen – Haptoglobin – α_1-acid glycoprotein – α_2-macroglobulin – C-reactive protein – Serum amyloid A – Albumin – mRNA transcription – Eukaryotic gene expression

The acute phase response represents an early and unspecific but highly complex reaction of the animal organism to a variety of injuries such as bacterial or parasitic infection, mechanical or thermal trauma, malignant growth or ischaemic necrosis. It includes not only the local reaction but also neurological, endocrine and metabolic alterations which are expressed as fever, leucocytosis, changes in the concentration of some heavy metals in blood and liver, activation of the clotting, the complement, kinin-forming and fibrinolytic pathways, transfer of amino acids from muscles to the liver followed by a drastic re-arrangement of plasma protein synthesis (for review see 10, 15, 21). At least some of the effects of the acute phase response are regarded as beneficial to the injured organism helping to restore disturbed homeostasis by checking bleeding, by demarcation and resorption of necrotic tissues, by binding and removal

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of excessive amounts of proteinases and exogenous substances, by mobilization of the immune system, and by preparing conditions for reparative processes and wound healing.

Acute phase proteins

The term "acute phase proteins" (AP-proteins) is generally used in reference to those plasma constituents whose concentration is significantly increased during the acute phase response. Fibrinogen, haptoglobin and α_1-acid glycoprotein belong to common AP-proteins in almost all mammalian species, while albumin is often decreased and so denoted as "negative" AP-protein. On the other hand, C-reactive protein (the first acute phase protein described) which rises dramatically in the blood of injured man or rabbit remains on a rather constant level in the blood of rats. The latter animal responds to injury be spectacularly enhanced synthesis of α_2-macroglobulin, a plasma proteinase inhibitor which is unaffected by the acute phase response in human patients. This species-dependent variability in the behaviour of individual plasma proteins following injury brings up the interesting question of how the synthesis of acute phase proteins is regulated. By now it has been established that almost all AP-proteins are synthesized in liver parenchymal cells. Although macrophages are capable of the formation of some components of the complement system [9], and lung macrophages [8] and blood granulocytes were shown to produce α_1-proteinase inhibitor [1, 35] the contribution of extrahepatic sites to overall production of AP-proteins appears to be negligible.

Mediators of the acute phase response

The problem of how local injury can swiftly and profoundly alter liver protein synthesis has been the subject of extensive studies for many years. Initially a range of macromolecules (lysosomal enzymes, cellular proteins) or other biologically active compounds (histamine, catecholamine, corticosteroids, kinins, prostaglandins) were suspected as specific liver stimulants [21]. In the early 1970's two independent groups of investigators: Pekarek, Wannemacher, Powanda and Beisel from Fort Dietrick (Maryland) and Kampschmidt and co-workers from Ardmore (Oklahoma) drew attention to leucocytes as the source of important mediators of the acute phase response. In a series of well-documented papers both these groups demonstrated that cells from glycogen-induced rabbit peritoneal exudates release a low molecular weight protein, leucocytic endogenous mediator (LEM), which after injection to rats or rabbits elevates body temperature, decreases serum iron and zinc, causes the release of neutrophils from the bone marrow and stimulates the flux of amino acids into the liver followed by enhanced synthesis of acute phase proteins [18, 42]. Subsequent studies of these and other authors pointed to similarities of LEM and other mediators produced by stimulated macrophages: endogenous pyrogen (EP), lymphocyte activating factor (LAF) and serum amyloid A inducer (SAA inducer) [19, 25, 40].

In the light of this evidence the principal macrophage-derived cytokine showing LAF activity has been renamed "interleukin 1", IL-1 [10, 29]. Although the name is

somewhat misleading, as IL-1 appears to be much more than a signal between various types of leucocytes and it may comprise several components, it has gained widespread acceptance. Oppenheim and Gery [29] defined IL-1 as "a macrophage-derived, hormone-like factor of mol. wt. 12,000–16,000 that has a multiplicity of pleomorphic amplifying effects on immunologic and inflammatory reactions. It is a genetically unrestricted, immunologically nonspecific factor which is active at low ($< 10^{-10}$M) concentrations". Experiments with partly purified IL-1 from various sources suggest that this cytokine may affect multiple target cells [10, 15, 29, 44]. In the hypothalamus Il-1 increases formation of prostaglandins and induction of fever; stimulation of leucocytes leads to release of lysozyme and lactoferrin; stimulation of thymocytes augments their interleukin-2-mediated proliferation; stimulation of T-lymphocytes enhances their response to mitogens and antigens and of B-lymphocytes augments antibody production; stimulation of muscle cells increases protein degradation by cathepsin-B-like proteinases; stimulation of fibroblasts elicits formation of prostaglandins and release of collagenase; stimulation of liver cells leads to enhanced uptake of amino acids, iron and zinc and to increased formation of acute phase proteins.

Since it is still not clear whether all the functions ascribed to IL-1 are indeed subserved by a single factor it may be better to use the terms LAF/IL-1 or EP/IL-1 depending on whether the biological activity is assessed in the thymocyte proliferation assay (LAF) or by inducing fever in vivo (EP). The situation is complicated by the fact that preparations of IL-1 obtained by various authors show considerable heterogeneity in respect of both charge and molecular weight: apart from the principal component of M_r approx. 15,000 there are reports on active high molecular mass (30,000–70,000 daltons) and low molecular mass species (4,000 daltons) and both acid (pI 4.5–5.0) and neutral (pI 7.0–7.3) proteins [11, 19, 26, 27, 44]. However, this controversy may soon be resolved since the IL-1 gene of both mouse [24] and man [2] has been cloned. Expression of the cloned murine gene in E. coli indicates that the protein is synthesized as a precursor of 270 amino acids (M_r 33,000) which is probably secreted and then proteolytically converted to lower mol. wt. forms. Variable proteolytic degradation may be responsible for the conspicuous molecular heterogeneity. The carboxyterminal 156-amino acid peptide is active not only in the thymocyte proliferation assay [24] but also stimulates synthesis of serum amyloid A protein and inhibits synthesis of albumin in cultured mouse hepatocytes [31], and stimulates synthesis of α_2-macroglobulin and inhibits synthesis of albumin in cultured rat hepatocytes [4].

Cellular sources of IL-1 and other cytokines

Initial experiments suggested that LEM/IL-1 derives from stimulated granulocytes but serious doubts were cast on this idea when Hanson et. al. [17] demonstrated that in a mixed cell population from rabbit peritoneal exudate the adherent mononuclear cells were the only source of EP. Since then numerous authors have demonstrated that activated blood monocytes and tissue macrophages are the principal source of LAF/IL-1 [cf. 10, 15]. In addition, LAF/IL-1 is produced constitutively by certain established cell lines of not only macrophage origin [23, 43] but also of keratinocyte origin [7, 13, 37]. These cell lines, often after additional stimulation or "superinduction",

have recently been used for purification of LAF/IL-1. On the other hand, superinduction may completely eliminate synthesis of cytokines which stimulate hepatocytes [43].

Hepatocyte cultures in the studies of AP-proteins

Assessing the effect of cytokines on the synthesis of acute phase proteins in vivo is difficult because of the complexity of the system. The primary hepatocyte culture in a well-defined medium offers a clear advantage and has been recently used by several authors [3, 6, 12, 25, 31, 33, 34, 41, 43]. We examined the effect of cytokines derived from human and mouse cells on the production of several plasma proteins by cultured mouse, rat and human liver cells [6, 22]. Conditioned tissue culture supernatants from stimulated blood monocytes, unstimulated COLO-16 cell line and peritoneal mouse macrophages were dialyzed, concentrated and subjected to molecular sieving on a Sephadex G-100 column. The collected fractions were assayed for LAF activity in the thymocyte proliferation assay (incorporation of ^{3}H-thymidine by concanavalin-A-stimulated cultured mouse thymocytes) and for hepatocyte stimulating factor (HSF). The term HSF was introduced by Fuller and co-workers who observed increased synthesis of fibrinogen by rat hepatocytes cultured for 2–3 days with supernatants of human monocytes [34] or rat liver Kupffer cells [36]. In our experiments we found that human-cell-derived cytokines stimulated rat hepatocytes to enhanced production of not only fibrinogen but also α_2-macroglobulin and α_1-AP-globulin while at the same time synthesis of albumin was significantly depressed [6, 22]. The ratio of α_2-macroglobulin: albumin synthesis was found to be the most sensitive indicator in the HSF bioassay with cultured rat hepatocytes [23]. Using this bioassay we found that the hepatocyte stimulating factor from human monocytes was eluted from Sephadex G-100 in fractions corresponding to 30,000 daltons as opposed to lymphocyte activating factor which eluted as a molecule of approximately 15,000 daltons (or in some cases also 45,000 daltons). Moreover, after column chromatofocussing the Sephadex-purified cytokine from human monocytes was resolved into 3 distinct peaks (pI 6.9, 5.5 and 5.1) with respect to LAF activity whereas the hepatocyte stimulating activity eluted at pH 5.1 [22]. These observations suggest that the lymphocyte activating factor and the hepatocyte stimulating factor in the preparations from human monocytes may represent different molecular entities. Such a conclusion is supported by recent results of Woloski and Fuller [43] who observed stimulation of fibrinogen synthesis in the cultures of rat hepatocytes by cytokines isolated from leukaemia cell lines. The preparations were virtually devoid of LAF activity and showed molecular mass in the range of 25,000–70,000 daltons. Baumann and co-workers [7] partly purified from COLO-16 cell line hepatocyte stimulating factors (M_r 30,000–70,000) that increased synthesis of α_2-macroglobulin, α_1-acid glycoprotein, α_1-antichymotrypsin and haptoglobin in cultured rat hepatocytes. These findings are not easy to reconcile with the fact discussed above that cloned murine LAF/IL-1 also stimulates mouse and rat hepatocytes [4, 31]. Although the relationship between HSF and LAF/IL-1 requires further studies it is tempting to speculate that the two cytokines either derive from a common precursor or, as in case of interferon [45], are products of distinct but related genes. Multiple molecular forms of cytokines from the IL-1 family might be very useful for an injured organism in the fine tuning of the acute phase response.

Mechanism of action of IL-1 related cytokines on target cells and regulation of acute phase protein synthesis

On the analogy of many cell growth factors and of protein hormones it is to be expected that IL-1 will be recognized by a specific receptor on the plasma membrane of target cells. So far such a receptor has not been identified although some indirect evidence suggests its presence, at least on cells of lymphocytic origin. Thus LAF/IL-1 can be absorbed from the medium by live and glutaraldehyde-fixed 1A5 cells [14]. Also the search for cellular second messengers for IL-1 has been rather unsucessful and the once suspected cyclic nucleotides have been excluded [29]. Dinarello [10] suggests that rapid accumulation of intracellular calcium induces changes that are indistinguishable from those of IL-1 activity, at least in some target cells. Prostanglandins of the E series are implicated in stimulation of the hypothalamus and induction of fever, as well as in increased muscle protein breakdown or release of collagenase by fibroblasts [10, 15] but they have no effect on protein synthesis by hepatocytes [39].

Whatever may be the mechanism of HSF/IL-1 action on liver cells cytokines ultimately affect the abundance of specific mRNAs coding for individual proteins. Several authors have demonstrated that following turpentine injection in vivo [28, 30, 32] or cytokine treatment in vitro [6, 31] rat and mouse hepatocytes contain a higher number of mRNA copies of "positive" AP-proteins such as α_1-acid glycoprotein, fibrinogen, α_2-macroglobulin or serum amyloid A protein, and a reduced number of mRNA copies of "negative" AP-proteins, such as albumin. Activation of AP-protein genes probably occurs by interaction of regulatory molecules with the promoter region located on the DNA strand upstream of the initiation codon [15]. This suggestion is based on recent advances in the structure and expression of other eukaryotic genes such as that of human metallothionein [20]. It is tempting to speculate that promoters of all AP-proteins contain common regulatory sequences capable of recognizing HSF/IL-1, or its putative second messenger. Binding of HSF to these regions would then enhance transcription of the genes responsible for positive AP-proteins, or reduce expression of genes coding for negative AP-proteins. In addition, the promoters may also contain regulatory sequences recognized by hormones and other modulators. Such an organization of the genes coding for AP-proteins would explain the permissive effect of glucocorticoids in the acute phase response in the rat [3, 21, 22, 42], or even the direct induction of these proteins by dexamethasone independently of HSF/IL-1 [5, 6, 16]. Moreover, by assuming that the regulatory elements can be reshuffled within the genome in the course of evolution the considerable species-related variability becomes more comprehensible. In the light of the swift progress of molecular biology it is likely that the fine structure of genes coding for major acute phase proteins and the mechanism responsible for their expression will be elucidated in the near future.

Acknowledgements. Work in the authors's laboratory on acute phase proteins was supported by a grant from the Polish Academy of Sciences (Project II.1.2.2 co-ordinated by the Nencki Institute, Warsaw) and by Grant P-05-111-N from the National Institute of Health, USA, through the M. Sklodowska-Curie Fund established by contributions from the United States and Polish Governments (U.S. Consulting Scientist: Dr. A. M. Chandler).

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Received February 11, 1985/Accepted June 21, 1985