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An alternative regulatory pathway of the acute phase response: the role of fibroblast-derived interferon-β_2

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Bacterial infection and tissue injury both lead to a series of adaptive homeostatic reactions known collectively as the acute phase response. This includes fever, leucocytosis, increased secretion of glucocorticoids, transfer of amino acids from muscles to the liver, and drastic changes in synthesis of certain plasma proteins (acute phase reactants) such as fibrinogen (FBG), haptoglobin (HPT) and α_1-acid glycoprotein (AGP)[1]. Most of the phenomena of the acute phase response are attributable to the action of inflammatory cytokines, a group of low molecular weight proteins produced by monocytes and named originally leucocytic endogenous mediator or endogenous pyrogen, but more recently shown to be mixtures containing interleukin-1 (IL-1), tumor necrosis factor (TNF) and some other hormone-like factors[2, 3]. Because of the complexity of the intact organism, experiments in vivo cannot be expected to indicate unequivocally which of the cytokines are responsible for induced synthesis of acute phase proteins. However, recent work with primary cultures of rat and mouse hepatocytes or established lines of human hepatomas have shown that liver cells are stimulated to some extent by purified or recombinant IL-1 and TNF[4, 5]. Although active, these cytokines do not elicit synthesis, in the same system, of the whole range of acute phase proteins, as occurs when conditioned media from endotoxin-stimulated monocytes-macrophages are used[6, 7]. These observations strongly supported the existence of a separate hepatocyte stimulating factor (HSF) (or factors), as postulated earlier by several authors[8–12]. It has become clear that HSFs are distinct from IL-1 and TNF, and are produced not only by monocytes-macrophages but also by some leukemic cell lines[13], by keratinocytes and by human squamous carcinoma cells[10, 11]. The precise nature of any type of HSF remained unknown until, recently, Gauldie and co-workers[13] discovered that interferon-β_2 (IFN-β_2) produced by human fibroblasts elicits a strong acute phase response in cultured liver cells, thus qualifying as yet another HSF, or the main hepatocyte stimulating factor. The properties of IFN-β_2 appear to be very similar to monocyte-derived HSF. Despite its name IFN-β_2 shows rather low antiviral activity. However, because it enhances growth of B-lymphocytes and murine B-cell hybridoma and plasmocytoma it has also been named BSF-2[15–17].

In the experiment described by Gauldie et al.[14] human hepatoma cells Hep G 2, or rat hepatocytes as a monolayer, were cultured for 2 days with the addition of conditioned media from human peripheral blood mononuclear (PBM) cells, from human lung fibroblasts, or with preparations of human recombinant IL-1 β and human recombinant IFN-β_2. The typical acute phase proteins secreted by the hepatocytes or the hepatoma cells were determined by electroimmunoassay as described previously[9].

As shown in the table, conditioned media from mononuclear cells and fibroblasts, as well as Hr IFN-β_2, stimulated production of all those acute phase proteins tested, including

Stimulation of acute phase protein synthesis by human hepatoma cells HepG 2 treated with human cytokines [14].
The cultured cells were exposed to dialyzed supernatants of LPS-stimulated human peripheral blood monocytes (PBM-CM), cultured human lung fibroblasts (Fibroblast-CM), human recombinant IL-1 β or human recombinant IFN-β_2 and after 2 days acute phase proteins were determined by electroimmunoassay in the hepatoma media.

Hepatocyte treatment	Protein secreted (μg/24 h × 10^6 cells)			
	FBG	ACT	AGP	HPT
Control	0.1	1.3	0.3	0.4
PBM-CM (1:20)	0.4	11.6	5.8	2.2
Fibroblast-CM (1:10)	1.4	8.1	0.9	1.5
rIL-1 β (250 U/ml)	0.01	3.1	1.8	1.2
rIFN-β_2 (60 U/ml)	1.7	9.9	1.4	1.7

fibrinogen, but HrIL-1 β augmented mainly the formation of AGP and to a lesser extent of α_1-antichymotrypsin (ACT) and haptoglobin, whereas synthesis of FBG was inhibited. In rat hepatocytes cultured in the presence of dexamethasone, HrIFN-β_2 stimulated synthesis of α_2-macroglobulin and cysteine proteinase inhibitor but suppressed synthesis of albumin, which is known as a 'negative' acute phase protein. These results have already been confirmed by Heinrich and co-workers [18] who demonstrated that human rIFN-β_2 elevates fibrinogen mRNA and decreases albumin mRNA in rat hepatoma Fao-9 cells.
The effectiveness of HrIFN-β_2 as a stimulator of the acute phase response was further demonstrated by the use of antisera to human fibroblast interferon. The first of these antisera had activity against both IFN-β_1 and IFN-β_2 and removed all HSF activity for fibrinogen from the PBM supernatant. Since the second antiserum with activity restricted to IFN-β_1 failed to affect acute phase protein synthesis, the importance of IFN-β_2 as a stimulator of hepatocytes was again indicated [14].
These recent findings have demonstrated the role of fibroblast-derived IFN-β_2 in relation to the acute phase response. However, the identity of HSF/IFN-β_2 produced by human fibroblasts and human monocytes and other cells has yet to be proved by the sequencing of purified cytokines or by cloning the corresponding genes. Also, future studies will be necessary to ascertain the relative contributions to the acute phase response of IFN-β_2 and other hepatocyte stimulating factors derived from macrophages and human squamous carcinoma cells. That in certain circumstances IL-1 may have little or no effect was shown earlier [7] and has been confirmed more recently by means of the antisera to IL-1 which failed to reduce the activity of PBM supernatant in stimulation of synthesis of α_2-macroglobulin or cysteine proteinase inhibitor by rat hepatocytes [14].
Discovery of the hepatocyte stimulating activity of IFN-β_2 changes current views on the regulation of the liver acute phase response, shifting the emphasis away from monocytes as the main source of inflammatory cytokines. Instead, the importance of fibroblasts and the alternative regulatory pathway with IFN-β_2 has to be taken into account. It appears that although this cytokine regulates B-cell function the hepatocytes are also another of its main targets.
Some information is already available in respect of the specificities of different acute phase stimulators. Thus in mouse hepatocytes IL-1 stimulates mainly serum amyloid A protein, and complement factors B and C 3, simultaneously suppressing albumin synthesis [4], and in rat cells IL-1 and TNF affect only AGP and albumin [7]. Furthermore, these cytokines have broader effects in human hepatoma cells [5, 6]. The addition of recombinant IL-1 or TNF to the conditioned medium from rat peritoneal macrophages blocks the induced synthesis of cysteine proteinase inhibitor by cultured rat hepatocytes [7]. These findings suggest the occurrence of reciprocal modulation of the acute phase response by individual inflammatory cytokines depending on both absolute concentrations and relative proportions of IL-1, TNF and HSF in the hepatocyte medium.
We conclude that the pattern of acute phase proteins synthesized by liver cells is genetically programmed and is adjusted in detail by the concerted action of various inflammatory cytokines. It appears that IFN-β_2 is the main signal from fibroblasts and certain other cells its production being stimulated by IL-1 and TNF [15, 17] derived from monocytes. In this way the organism may achieve the most effective homeostatic responses to infection and inflammation and make use of the individual properties of the acute phase proteins.

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