LPS stimulation[24] and the known importance of IL-6 and PGE_2 on *in vitro* hepatocyte proliferation indicate the central importance of the Kupffer cell in this unique mammalian response.

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Discussion

DR. ROBERT ZEPPA (Miami, Florida): Dr. Bland, Dr. Jones, colleagues, I am indebted to Dr. Flye for his invitation to discuss this paper under the assumption that I know something about cytokines. That is in error. I think Wayne asked me to comment on this because 20 years ago Joe Levi and I were studying the regenerative problem of rodent liver. And, of course, in paired *in situ* perfused animals, we discovered that at 24 hours, as Dr. Flye has pointed out, there came into the circulation media some substance that we were never able to characterize, which had an effect on normal liver if they were cross-circulated, in that DNA synthesis went up at that 24-hour period in both livers. That is by way of some historical background. But what I would like to ask Dr. Flye about these experiments is, if in fact the animals are pretreated with indomethacin, what happens then to your pellet of Kupffer cells? This is fascinating and reminds me a little of the presentation that Dick Simmons gives in which he calls it "pillow talk in the liver," that is, the communications between the Kupffer cells and the hepatocytes, which appear now more and more, thanks to the work of Dr. Flye and his group, to be one of the most important factors that we have in terms of how the liver responds to a variety of stimuli, not merely regeneration. Thank you.

DR. COURTNEY M. TOWNSEND, JR. (Galveston, Texas): Dr. Bland, Dr. Jones, Fellows and Guests, Dr. Flye and his colleagues have long been interested in the problem of hepatic regeneration and the interaction of immunologically competent cells with hepatocytes. This study, I think, provides more information into the mechanisms by which Kupffer cell functions are regulated. It appears that there is a tight autocrine control, at least of prostaglandin, on interleukin-6 (IL-6) production.

What are the signals that are at play here? That is, what turns the Kupffer cells on to begin increasing their responsiveness to make IL-6 in response to lipopolysaccharide? Also, does a reciprocal relationship exist? That is, if you were to decrease IL-6, would you increase prostaglandin production? And is there any evidence that exogenously added prostaglandin would further affect the level of IL-6 produced? And, finally, do you know whether the mechanism of action of prostaglandin on IL-6 production is direct or indirect? And if it is indirect, what possible second messengers are involved? Thank you very much.

DR. GEORGE PARKER (Richmond, Virginia): Dr. Bland, Dr. Jones. Like Dr. Zeppa, I am not sure why I was asked to discuss this paper. I think the only reason is that 19 years ago Wayne Flye was my first senior

resident when I was an intern, and he still treats me the same. I have a couple of questions and a couple of comments.

The comment would be I think it is a very elegantly designed and carried out study with all the proper controls, so I think the data are incontrovertible.

The first question I would have is, do you have any idea if the amount of interleukin-6 that is being produced would be sufficient to induce hepatocyte stimulation and mitosis? Second, you mentioned about the factors of the Kupffer cell inducing regeneration or helping in the regenerative process. What is different about certain injuries where there is just fibrosis *versus* certain injuries where there is regeneration? And would you speculate on the possible role of the Kupffer cell there? Thank you very much.

DR. WAYNE FLYE (Closing discussion): I would like to thank the discussants. Dr. Zeppa, you and Dr. Bernard Fisher earlier performed pioneering work in liver regeneration. Obviously, this is a much more complex phenomenon than can simply be explained by the effect of cytokines. Cytokines appear to regulate, more than initiate, regeneration. If you add PGE_2 to hepatocytes in culture, you can get increased DNA proliferation. Addition of indomethacin blocks prostaglandin synthesis and retards the course of liver regeneration. High doses of PGE_2 in vivo will result in diarrhea and animal death.

Dr. Townsend, you ask what signals are important for regeneration. In vitro addition of interleukin-6 downregulates prostaglandin E_2 production, as does tumor necrosis factor and interleukin-1. This may be effected by activation of phospholipase A2 and the mobilization of fatty acids and arachidonic acid from the cell surface. We know that PGE_2 is an important regulator of cell proliferation, probably by increasing cyclic adenosine monophosphate (cAMP) levels. When we infuse the long-acting 16,16 dimethyl PGE_2, which is not metabolized on first passage through the lung, as are most natural prostaglandins, into a heterotopic heart allograft continuously for 2 weeks, we completely prevent graft rejection. We know that the early recognition phase of rejection is related to how the macrophage presents antigen to effector lymphocytes. We have evidence that the migration of lymphocytes and macrophages through the graft are impaired by PGE_2. Although liver regeneration is a different phenomenon, the mechanism of regulation of second signals is probably similar. Changes in interleukin-6 levels do not cause changes in PGE_2 production, whereas exogenous PGE_2 does decrease interleukin-6 levels.

Although the Kupffer cells are probably normally exposed to low levels of endotoxin, it is only during exposure to higher levels that Kupffer cells significantly increase PGE_2 and cytokine production, *i.e.*, during bacteremia and during the stress of liver regeneration. However, R. P. Cornell has shown that neutralization of the lipid A portion of endotoxin with polymyxin B in normal animals slows liver regeneration. The levels of PGE_2 produced by the Kupffer cells appear adequate to regulate the adjacent hepatocytes.

Dr. Parker, the fibrotic liver does not regenerate well. This is clinically important in the patient with cirrhosis undergoing liver resection. The hepatocyte within the milieu of the fibrotic tissue behaves differently than when it can function in a normal hepatic environment.

I would like to thank the discussants for their remarks and the Association for the privilege of presenting this work.