Other Microbial Components Associated with Hepatitis C Virus Infection: Their Effects on Interferon-α/Ribavirin Treatment

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Introduction

The hepatic immune response in patients with hepatitis C virus (HCV) infection has been the object of intensive studies. In fact, HCV is a lymphotropic virus and CD81 acts as its co-receptor on CD4+, CD8+, CD19+, and CD56+ lymphocytes, respectively (Kronenberger et al., 2006). Generally, antigen (AG)-specific CD8+ cell cytotoxic response is very effective in viral clearance, being that this function is sustained by the intervention of CD4+ cells via release of interferon (IFN)-γ and interleukin (IL)-2, which expands the pool of T cytolitic cells (Jellison et al., 2005). Additionally, activated AG-specific CD8+ cells generate a pool of memory cells, which protect the host against subsequent infections (Jabbari & Harty, 2006). However, this seems not to be the case in HCV infection because of mutations within immunodominant CD8 epitopes, which allow HCV to escape from immunosurveillance (Chang, 1998; Urbani et al., 2005). On the other hand, early defects of CD8+ cell cytolytic function have been reported in HCV infection, even including the capacity of these cells to exert antiviral activity (Rehermann & Nascimbeni, 2005; Bowen & Walker, 2005). In fact, in this precocious phase, CD8+ T cells interacting with high avidity with AG-presenting cells (APCs) may undergo deletion or poor activation (Bowen et al., 2005). On the other hand, later in infection CD8+ cells activated by HCV in the lymph nodes (LN) may interact with lower avidity with APCs, thus leading to an ineffective viral clearance (Snyder et al., 2003). Taken together, these events seem to contribute to HCV disease chronicity. Conversely, CD4+ cells seem to be very active against some conserved protein epitopes of the HCV, and their response correlates with viral clearance (Harcourt et al., 2004). Either in HCV-infected patients or in HCV-infected chimpanzees (Shoukry et al., 2004), production of IFN-γ from T helper(h)-1 cells is associated with a rapid clearance of circulating HCV.

Consequently, development of chronicity in HCV-infected patients may rely on a defective HCV AG presentation to T cells and, therefore, on a failure to maintain and sustain a robust Th1 response against immunodominant proteins (Neumann-Haefelin et al., 2005).

In HCV infection, AG presentation is strongly supported by dendritic cells (DCs), and *in vitro* studies have clearly demonstrated that human DCs expressing

HCV core and NS3 AGs were able to activate T cells to proliferate and release cytokines (Li et al., 2006).

Of note, early AG presentation in the LN during HCV infection abolishes intrahepatic tolerance, leading to a more efficacious cytotoxic immune response. In this framework, it is worth mentioning the role played by NKT cells, a T cell subset specific for CD1d, predominantly present in mouse liver and also in human liver chronically infected with HCV (Sandberg & Liunggren, 2005). Quite interestingly, NKT cells arise in the thymus and are positively selected via interaction with MHC class I molecules on double-positive CD4+ CD8+ thymocytes (Sandberg & Ljunggren, 2005). As far as the trafficking pattern of these cells is concerned, NKT cells express a chemokine receptor profile very similar to that of Th1 inflammatory cells (Godfrey & Kronenberg, 2004). Functionally, the interaction between liver DCs and NKT cells leads to a dramatic release of IFN-γ and IL-4 in the latter (Trobonjaca et al., 2001). This event might suggest the role played by NKT cells in the initiation and regulation of the immune response.

Just recently, the role of the chemokine receptor CCR5 has been emphasized as an important modulator of the inflammatory response in the course of HCV infection (Ajuebor et al., 2006). In fact, interaction of CCR5 with its intrahepatic ligands favors the recruitment of Th1 cells into the liver, thus promoting clearance of HCV during acute infection (Boisvert et al., 2003). Quite interestingly, it seems that IFN-α possesses the ability to increase expression of CCR5 on T cells during HCV infection, thus contributing to viral clearance (Yang et al., 2001).

However, further data are required to confirm whether downregulation of CCR5 expression on T cells renders individuals more susceptible to HCV infection.

In HCV infection, B lymphocytes are present intrahepatically and harbor HCV (Sansonno et al., 1998). Under HCV antigenic pressure, B cells undergo clonal expansion often associated with generation of rheumatoid factor, which, in turn, interacts with human MHC class I AGs, thus interfering with peptide recognition by T cells (Williams et al., 1994). With special reference to auto-antibodies, antilactoferrin (LF) auto-antibodies have been detected in HCV+ patients and, mostly, in nonresponders to IFN-α/ribavirin treatment (Amati et al., 2004). In particular, LF is an iron binding protein endowed with antiviral activity and able to bind to the lipid A moiety of bacterial endotoxins (Caccavo et al., 1999, 2002). Therefore, production of anti-LF auto-antibodies in HCV disease may aggravate its clinical course by depotentiating the anti-inflammatory activities of LF.

Finally, the lack of correlation between a strong Th response and a corresponding robust antibody response in HCV-infected patients may depend on the attitude of CD4+ cells to promote CD8+-dependent cytotoxicity rather than immunoglobulin production (Napoli et al., 1996; Cacciarelli et al., 1996).

As far as hepatic innate immunity is concerned, Kupffer cells (KCs) represent the largest contigent of resident macrophages (MØ) present in the body (Fax et al., 1989). KCs are able to capture and present AGs and express the co-stimulatory CD80 and CD86 molecules (Burgio et al., 1998). In contrast to DCs, KCs do not migrate out of the liver and present AGs locally (MacPhee et al., 1992). MHC-I-positive hepatocytes, despite the lack of co-stimulatory molecules (Ni et al., 1999),

can directly present AGs to uncommitted T cells via ICAM-1 (Bertolino et al., 1998). KCs are able to recognize AGs via Toll-like receptors (TLRs) with a subsequent release of pro-inflammatory cytokines and oxygen free radicals (Liu et al., 1998). Therefore, in the course of HCV infection, hyperactivation of KCs can cause further liver damage through the release of the above-cited mediators (Fearns et al., 1995). However, a mechanism of hepatic immune tolerance has been established by liver sinusoidal endothelial cells (LSECs) (Knolle & Limmer, 2001). They behave as APCs and resemble immature DCs, which are resistant to maturation even under tumor necrosis factor (TNF)-α and endotoxin stimulation. Functionally, LSECs attenuate Th1-mediated responses, thus facilitating antibody response. Furthermore, as far as intrahepatic tolerance is concerned, DCs express IL-10 in the liver, thus rendering APCs tolerogenic (Goddard et al., 2004). On the other hand, the role of NKT cells in intrahepatic tolerance is still debated (Godfrey & Kronenberg, 2004).

Quite interestingly, polymorphonuclear cells (PMN) represent 1–2% of the total nonparenchymal cells found in normal mouse liver (Gregory et al., 1996). In *Listeria*-infected mice, a massive infiltration of immigrating PMN and their co-localization with KCs into the liver have been reported (Gregory et al., 2002). *Listeriae* organisms were phagocytosed by PMN and subsequently found within KCs. PMN and KCs interacted via adhesion molecules [CD11b/CD18(MAC-1) and CD54(ICAM-1)], and, finally, adherent PMN were ingested and lysed by KCs. This mechanism can also be interpreted as an attempt by KCs to decrease the release of inflammatory mediators by activated PMN. The intrahepatic role of PMN has also been demonstrated by experiments of neutrophil depletion in mice that led to an accumulation of various bacteria previously inoculated intravenously (IV) (Verdrengh & Tarkowski, 1997; Van Andel et al., 1997; Conlan, 1997).

Concurrent Effects of Bacterial Endotoxins in the Course of HCV Infection

Bacterial endotoxins or lipopolysaccharides (LPS) from the outer cell membrane of gram-negative bacteria are deeply involved in the pathogenesis of sepsis (Opal et al., 1999). For cell activation to occur, LPS interact with CD14-bearing inflammatory cells [monocytes (MO)-MØ, PMN, and endothelial cells] and through TLR-4 lead to the release of a plethora of pro-inflammatory cytokines, free radicals, platelet activating factor, complement components, tissue factor, and various noxious mediators (Wright et al., 1990; Poltorak et al., 1998).

Evidence has been provided that TLRs require accessory molecules for microbial recognition. In the case of LPS binding to TLR-4, LPS binding protein (LBP), CD14, and MD-2 play specific roles in that LBP and CD14 determine the magnitude of LPS responses and type I IFN production (Miyake, 2006). On the other hand, MD-2 is responsible for ligand binding and receptor activation (Miyake, 2006). Also, in the case of TLR-2, intervention of similar accessory molecules is needed.

All these harmful substances participate in the generation of the systemic inflammatory response syndrome, and the liver is the principal organ devoted to LPS detoxification (Jirillo et al., 2002). Experimentally, IV-injected LPS are taken up by hepatocytes, which seem to be involved in the clearance of these bacterial products by virtue of CD14 and TLR-4 expressed on their membrane (Vodovotz et al., 2001). Moreover, as a result of LPS injection into rats, endotoxins have been found in the bile and then excreted into the gut (Maitra et al., 1981).

In experimental hepatitis the major mediator involved in the liver damage is represented by tumor necrosis factor (TNF)-α, as demonstrated in mice treated with D-galactosamine (D-GalN) (Galanos et al., 1979). D-GalN increases the sensitivity of mice to LPS, and mortality occurs via a massive apoptosis of hepatocytes (Mignon et al., 1999). In this regard, the role of Fas ligand (FasL) in the induction of TNF-α-mediated liver apoptosis is quite controversial. In fact, in the LPS-D-GalN model, a defective Fas or a lack of functional Fas could not prevent mortality and liver damage (Tannahil et al., 1999). Instead, this was the case in the model of *Corynebacterium parvum* LPS, where blockade of FasL with soluble Fas fusion protein was protective in mice (Kondo et al., 1997).

Another mediator able to cause liver injury in response to LPS is IL-18 released by KCs. In the murine model, *Propionibacterium acnes*/LPS-induced liver injury, IL-18 induced Fas-dependent hepatocyte apoptosis via natural killer (NK) cell-induced increase of FasL (Tsutsui et al., 2000). This described model of acute liver damage is similar to that of fulminant hepatitis in mice with a gene transfection of FasL *(*Li et al., 2001). Conclusively, this pathogenic mechanism may serve to elucidate some aspects of the fulminant hepatitis described in the human HCV infection (Vento, 2000).

Taken together, the above data indicate that in experimental hepatitis, liver damage occurs through apoptosis and caspases seem to mediate cell death (Van Molle et al., 1999). Treatment with α1-antitrypsin may represent an anti-apoptotic mechanism, thus indicating that acute-phase proteins are able to prevent caspase activation (Van Molle et al., 1999).

Finally, a role has been attributed to LPS in ethanol-induced liver injury. There is evidence that ethanol increases the intestinal permeability to LPS (Enomoto et al., 1998) and, at the same time, antibiotics and lactobacilli treatments mitigate ethanol-dependent hepatic damage by reducing gram-negative intestinal flora (Adachi et al., 1995; Nanji et al., 1994). In addition, ethanol chronically administered to CD14 knockout mice generates less damage than that observed in the wild counterpart (Adachi et al., 1994). On the other hand, ablation of KCs by gadolinium chloride attenuates ethanol-mediated liver injury since these cells are the source of pro-inflammatory cytokines in response to LPS (Adachi et al., 1994). Finally, anti-TNF-α monoclonal antibody treatment (Iimuro et al., 1997) or the use of receptor-1 knockout mice (Yin et al., 2001) prevents hepatic damage caused by ethanol, thus further supporting the role of LPS in this pathology.

In humans, the presence of endotoxins in liver disease has been documented in many reports. Several authors have found endotoxemia cirrhotic patients, and it seems that amounts of LPS progressively augment as liver function deteriorates

(Nolan, 1975; Liehz et al., 1976; Prytz et al., 1976). This last finding may be predictive of short-term survival in cirrhosis (Chan et al., 1997). Furthermore, the evidence for a reduced phagocytic activity of KCs in cirrhosis may be the cause of endotoxemia in this clinical condition (Kuratsune et al., 1983). In this framework, it should be mentioned that postoperative hepatic failure in cirrhotic patients seems to be the result of an exaggerated release of pro-inflammatory cytokines by primed MØ activated by LPS spilling over in the blood during hepatic resection (Sato et al., 1997).

In other related studies conducted in HCV patients, a correlation was found between endotoxemia and elevated levels of serum CD14 (Jirillo et al., 1998). Similar findings were also reported in the case of alcoholic cirrhosis and HBV infection (Oesterreicher et al., 1995).

Over recent years, a number of investigations have attempted to clarify the origin of endotoxemia in the course of HCV infection. Quite interestingly, in HCV infection a defect of innate immunity has been described in terms of the reduced ability of phagocytosis and killing exerted by PMN and MO (Jirillo et al., 1995, 1996). Moreover, T-cell-mediated antibacterial activity was also impaired in these patients as a further demonstration of natural immunity depression (Jirillo et al., 1995, 1996). Therefore, gram-negative bacteria can gain easier access into the HCV host with subsequent liberation of endotoxins at systemic or tissue levels. At the same time, the altered architecture of the liver can reduce its detoxifying capacity, thus leading to the accumulation of various toxic products into the host, even including LPS (Jirillo et al., 2000). In relevance to this event, bacterial toxins of intestinal derivation, accumulated in the HCV-infected liver because of a putative altered intestinal permeability, may further aggravate the hepatic damage *(*Jirillo et al., 2000).

These data are in agreement with a recent view according to which activation of the innate immune system in the liver abrogates APC tolerance, thus avoiding T cell apoptosis (Bowen et al., 2005). Consequentially, increased survival of CD8+ cells gives rise to a more efficient intrahepatic cytotoxicity.

On these grounds, our group has conducted a series of investigations on the putative effects of endotoxins in HCV patients receiving 6 months' treatment with IFN-α/ribavirin (RIB) (Amati et al., 2002; Caradonna et al., 2002). Before therapy (T0), HCV individuals were subdivided into two groups—endotoxemic and nonendotoxemic—in order to evaluate the influence of LPS on their immune status. Thus, at T0, in endotoxemic HCV+ patients, absolute numbers of CD3+, CD4+, CD14+, and CD19+ cells were higher than those observed in the non-endotoxemic HCV+ counterpart.

Additionally, MO intracellular content of TNF-α and IL-1β was more elevated in endotoxemic patients than in non-endotoxemic ones under resting conditions. Following *in vitro* LPS stimulation of MO, in endotoxemic individuals values of these cytokines were even higher than in non-endotoxemic ones. The same group of patients, divided into responders and non-responders at the end of IFN-α/RIB treatment over a period of six months (T6), was immunologically re-evaluated. In responders, endotoxemia present at T0 was no longer detectable, while non-responders were still endotoxemic.-

Quite interestingly, in responders there was a parallel increase of serum levels of IFN-γ and IL-10, while in non-responders the increase in IFN-γ was not paralleled by an equivalent increase in IL-10.

Consequently, in non-responders the MO intracellular content of IL-1β and TNF-α was more elevated than in responders. Taken together, all these data suggest that in responders to IFN-α/RIB treatment a re-equilibrium between Th1 (inflammatory) and Th2 (anti-inflammatory) cytokines occurs. As a result of this balance, bacterial AGs, even including LPS, can be neutralized in a more efficient way by the effects of intestinal and hepatic phagocytes as well as of epithelial and liver endothelial cells. Binding and/or de-activation of LPS or enhanced phagocytosis of opsonized microorganisms seem to be the major immune mechanisms elicited in response to a successful treatment with IFN-α/RIB. In relevance to the above-described mechanisms, it has been hypothesized that in patients who resolve HCV infection depression of the innate immune response might not occur, thus leading to a breaking of intrahepatic tolerance (Rehermann & Nascimbeni, 2005). Resulting inflammatory status may be beneficial to the host in terms of HCV eradication.

In non-responders, the lack of anti-inflammatory activity exerted by IL-10 seems to be responsible for MO hyperactivation, as evidenced by the more elevated content of pro-inflammatory cytokines (IL-1β and TNF-α) in these cells. Similar findings have been reported in alcoholic cirrhosis, where a decreased release of IL-10 from MO in response to LPS has been discovered (Le Moine et al., 1995). Consequently, exaggerated secretion of TNF-α in alcoholic cirrhosis may be attributed to the observed lack of IL-10 production.

In HCV+ non-responder patients, hepatic and/or systemic oversecretion of pro-inflammatory cytokines aggravates liver damage. In particular, IL-1β *in vivo* is able to inhibit IFN-α/β-induced Stat1 tyrosine phosphorylation, thus hampering IFN-mediated antiviral activity (Tian et al., 2000). Conversely, in Stat1 knockout mice, IFN-dependent signaling pathways are absent, thus provoking a reduced antimicrobial immune response. According to these data, IL-1β may account for refractoriness to IFN-α therapy in HCV disease, thus representing a putative therapeutic target in this pathology (Diehl, 1999).

The Role of β-Glucans in the Course of HCV Infection

β-glucans (BG) are natural polysaccharides that represent normal components of the cell wall of fungi and bacteria, as well as of oats, barley, and yeast (Williams et al., 1998). BG are ubiquitously distributed in the environment and, therefore, living organisms possess pattern recognition molecules able to interact with these polysaccharides (Amati et al., 2005b). In fungi, in addition to polysaccharides, glycoproteins (mannoproteins) are also present, and, in particular, mannose residues can elicit a robust immune response into a susceptible host (Williams et al., 1998; Fraser et al., 2006). Here, emphasis will be placed on BG, whose ability to regulate immune response will be illustrated below.

As far as the interaction of BG with phagocytes and, in particular, with MØ is concerned, besides the MØ mannose receptor and the complement receptor 3, dectin-1 has recently been considered as the major MØ receptor for these molecules (Brown et al., 2002). LPS and BG activate MØ, both leading to an increase in NFKB (Underhill & Olinsky, 2002; Gantner et al., 2003). However, LPS utilize TLR-4 on MØ (Underhill & Olinsky, 2002), while BG activate MØ in TLR-4-deficient mice for the production of TNF-α (Kataoka et al., 2002). By contrast, in mice with a defect in the adapter protein MyD88, the BG-induced MØ response is lower than that observed in the wild-type murine counterpart (Marr et al., 2003). These data indicate that LPS and BG share some common postreceptorial pathways.

According to current literature, BG seem to be protective toward LPS-mediated toxic effects. In this respect, mice administered with BG before the induction of sepsis (cecal ligation and puncture) underwent less mortality than untreated animals (Williams et al., 1999). Actually, preadministration of BG correlates with less expression of TLR-2 and TLR-4 mRNA and less concentration of serum TLR-4. This mechanism of downregulation of LPS-induced NFKB activation could depend on BG-mediated inhibition of IKKβ kinase activity and altered phosporylation and degradation of IKβ-α (Williams et al., 2002).

In vitro studies with BG are quite controversial. In fact, it has been demonstrated that peripheral blood mononuclear cells and murine MØ, stimulated with BG, produced IL-1 and TNF-α (Abel & Czop, 1992; Seljelial et al., 1989). However, murine MØ pretreated with BG and then stimulated with LPS produced higher amounts of IL-6, while TNF-α production was suppressed (Soltys & Quinn, 1999). In general terms, according to studies in an *in vitro* human model, BG seem to promote production of IL-8 and IL-10 and to suppress IL-2 and IFN-γ release from Th-1 cells in response to endotoxins (Nakagawa et al., 20*0*3). Furthermore, it is worth mentioning that other investigations have emphasized the role of BG in the release of TNF-α from zymosan-activated MØ or in the upregulation of immunocompetent cell response, by their own or in synergy with LPS (Engstad et al., 2002). However, all the above discrepancies can be explained by the concentration of BG present in a given host. For instance, Hoffman et al. (1993) found that concentrations of BG less than 500 μg suppressed TNF-α release from rat alveolar MØ, while concentrations greater than 500 μg enhanced production of this cytokine in response to LPS.

In order to evaluate the effects of BG on the immune response in HCV patients undergoing IFN-α/RIB therapy, endotoxemia and β-glucanemia were measured in their blood at T0 and T6, respectively, as previously described in this chapter. Patients were subdivided into two subsets, LPS+/BG+ and LPS-/BG+, respectively, and then immune parameters were determined (Amati et al., 2005a). When serum levels of BG and plasma endotoxins were evaluated, endotoxemia, at T0, was detected in 22 of 46 patients, while BG were present in the sera of 44 of 46 patients. At T6, among 41 patients evaluated, endotoxemia was detected in 20 of them, while β-glucanemia was present in 38 individuals.

In terms of absolute numbers and percentage of lymphocyte phenotypes, no significant differences were observed between patients (at T0 and at T6) and normal donors. Quite interestingly, when patients were divided into two subsets, namely

LPS+/BG+ and LPS-/BG+ subjects, some interesting findings emerged. In particular, at T6 vs. T0, in the LPS-/BG+ subset there was an increase of CD3-CD8+ cells (a subset of NK cells) and of CD71+ cells (Figure 1), while memory cells (CD45RO+ cells) decreased (Figure 2). In the LPS+/BG+ counterpart, similar findings were not detected.

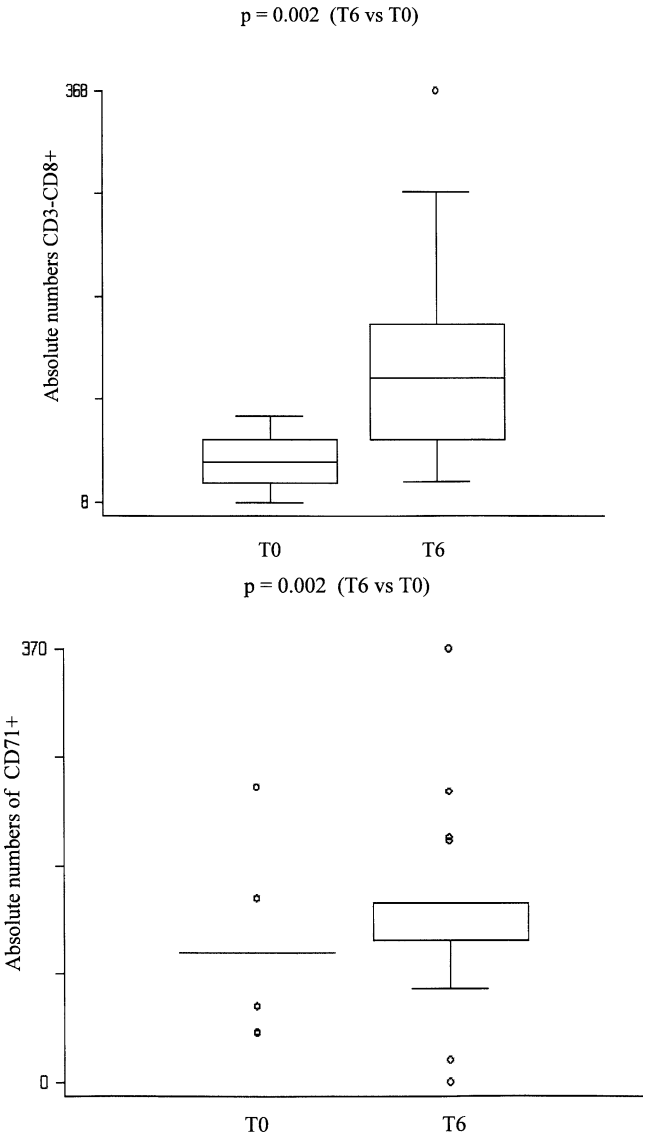
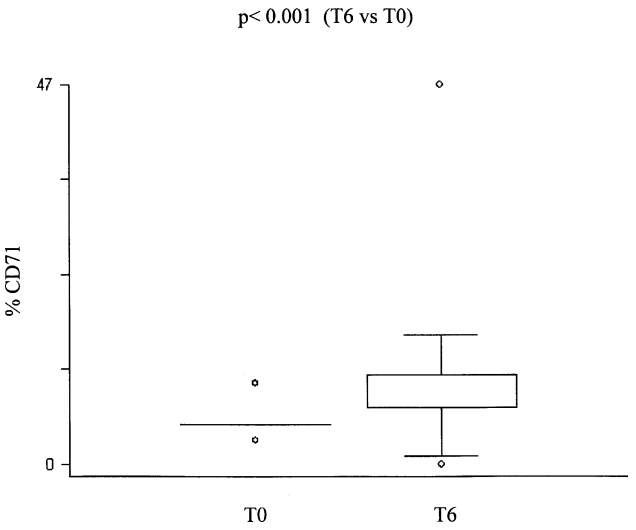


Fig. 1 Absolute numbers of HCV+ CD3-CD8+ cells (A) and of CD71+ cells (B). Samples from HCV+ LPS-/BG+ patients were analyzed at T0 and at T6, respectively, on a FACSCalibur [(Becton Dickinson Immunocytometry System, San Josè, CA (BDIS)] by cell surface staining with FITC/PE/FITC-conjugated monoclonal antibodies to CD3, CD8, and CD71 antigens, respectively



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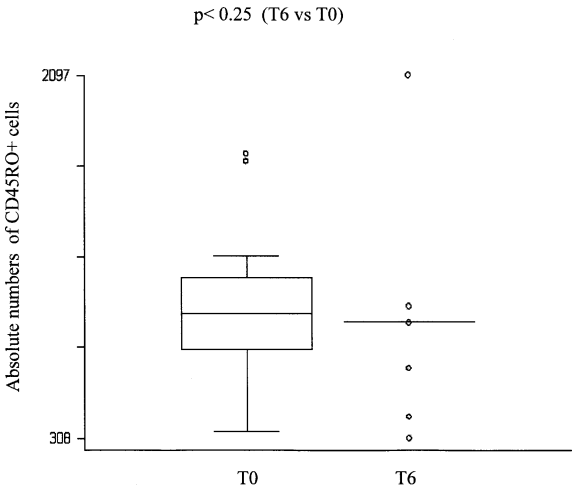

Fig. 2 Absolute numbers of HCV+ CD45RO+ cells. Samples from HCV+ LPS-/BG+ patients were analyzed at T0 and at T6, respectively, on a FACSCalibur (Becton Dickinson) by cell surface staining with FITC-conjugated monoclonal antibodies to CD45RO antigen

In another set of experiments, at T0, a correlation between LPS/BG levels and immune/enzymatic parameters was performed in LPS+ BG+ HCV+ patients. A negative correlation was found with CD25+ cells, gamma glutamyl transpeptidase (γ-GT) values, total bilirubin, and direct bilirubin. On the other hand, no significant correlations were found in the case of LPS-/BG+ patients. Furthermore, at T6, in the LPS+/BG+ patients a positive correlation was detected with CD3+ and CD4+ cells, glutamic-ossalacetic transaminase (GOT) and glutamil-piruvic transaminase (GPT) and direct bilirubin. Quite interestingly, at T6, in the LPS-/BG+ counterpart a positive correlation was determined with CD25+ (Figure 3) and CD95+ cells

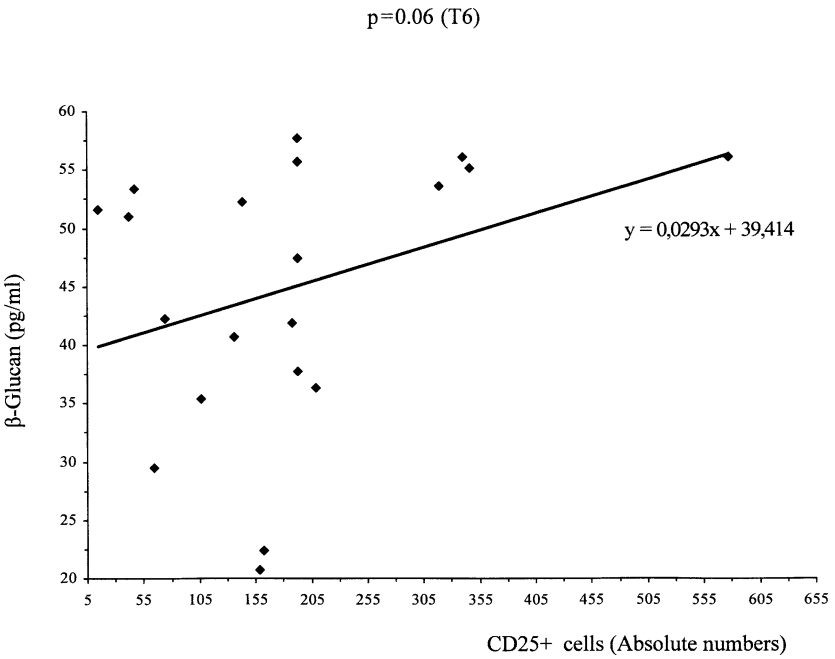

Fig. 3 Correlation between β-glucan serum concentration and absolute numbers of CD25+ cells, at T6, in LPS-/BG+ HCV+ patients. Spearman's rho = 0.42; p = 0.06

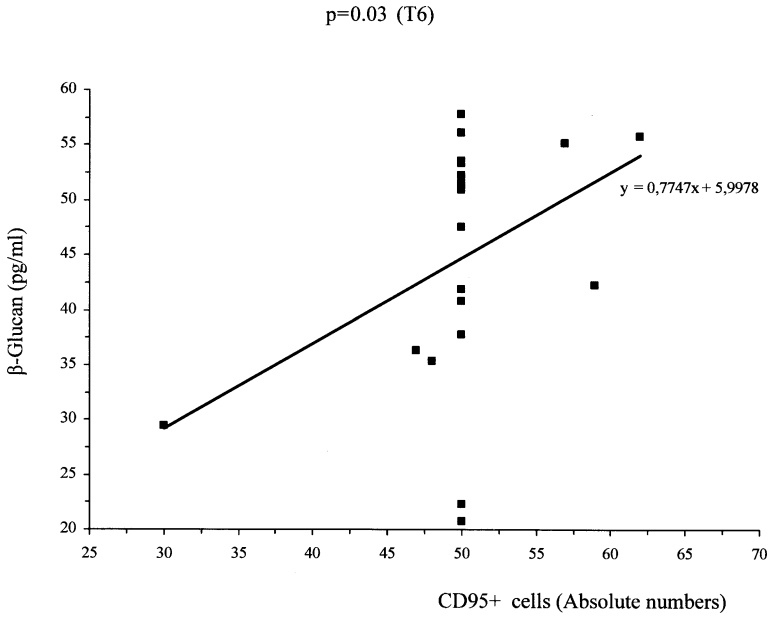

Fig. 4 Correlation between β-glucan serum concentration and absolute numbers CD95+ cells, at T6, in LPS-/BG+ HCV+ patients. Spearman's rho = 0.48; p = 0.03

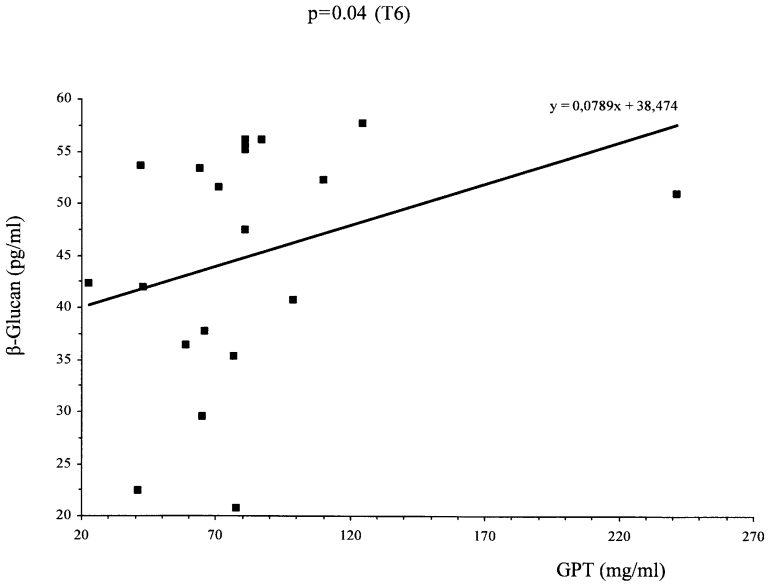

Fig. 5 Correlation between β-glucan serum concentration and GPT levels, at T6, in LPS-/BG+ HCV+ patients. Spearman's rho = 0.45; p = 0.04

(Figure 4) and GPT concentration (Figure 5), respectively. Conversely, a negative correlation was observed in the case of total bilirubin and direct bilirubin.

Conclusions

Taken together, the bulk of data reported in the previous sections clearly indicates the presence of both circulating endotoxins and BG in HCV+ patients before and after IFN-α/RIB therapy.

In the case of LPS, in responders endotoxemia is no longer detectable, while the status of a non-responder coincides with the presence of plasma LPS. On the other hand, at T6, levels of β-glucanemia are present in a percentage similar to that observed at T0. This suggests that therapy with IFN-α /RIB does not influence BG concentration.

The pathogenic mechanism accounting for the presence of circulating BG in HCV+ patients could be the same as that invoked in the case of endotoxemia. In fact, the impaired natural immunity in HCV disease, the reduced hepatic clearance exerted by KCs toward fungi and/or their components, and the increased intestinal permeability may represent important cofactors in the generation of glucanemia in an elevated percentage of patients (Amati et al., 2002; Jirillo et al., 1998).

As far as the role of LPS is concerned in HCV disease, our previous data have pointed out that, after IFN-α/RIB treatment in HCV+ patients, non-responders were

still endotoxemic while responders were no longer endotoxemic (Amati et al., 2002; Caradonna et al., 2002). Endotoxemia was associated with an increased content of IL-1β and TNF-α in MO and with an exaggerated production of NO (Caradonna et al., 2002). On the contrary, in responders the increased release of IL-10 led to an anti-inflammatory response that neutralized the production of pro-inflammatory cytokines (Caradonna et al., 2002). Conclusively, in non-responders the uncontrolled inflammatory process could aggravate liver damage (Amati et al., 2002; Caradonna et al., 2002).

To the best of our knowledge, here we have provided the first evidence on the relationship between β-glucanemia and liver function in HCV disease. In fact, at T6, in HCV+ patients who were LPS+/BG+, we have determined a positive correlation with GOT, GPT, and direct bilirubin serum levels, respectively. By the way, in the LPS+/BG+ patients, at T0, a negative correlation was found with levels of γ-GT and direct and total bilirubin. On the other hand, in the LPS-/BG+ subjects at T6, a negative correlation was detected with total and direct bilirubin, respectively. In addition, no correlation was found with GOT, while a positive correlation was determined with GPT only. Taken together, these data suggest that BG contribute to a lesser extent to the hepatic damage in the course of HCV disease, while LPS seem to exert more noxious effects on the liver (Jirillo et al., 2002). In this respect, evidence has been provided that intrahepatic DCs and LSECs are refractory to LPS effects (De Creus et al., 2005; Uhrig et al., 2005). Therefore, abrogation of LPS tolerance in HCV+ non-responder patients might contribute to disease progression. In addition, at T6, the presence of circulating BG, in the absence of LPS, is associated with an increase in CD3-CD8+ cells, a subset of NK cells, in CD71+ cells, and with a decrease in CD45RO+ cells, while positively correlating with CD25+ and CD95+ cells. By contrast, at T0, in LPS+/BG+ patients a negative correlation was found with CD25+ cells. Collectively, these findings allow us to formulate the following hypothesis. In HCV disease, BG seem to expand the pool of CD25+ cells and likely of CD4+CD25+ T regulatory (TREG) cells. TREG cells could exert a potent anti-inflammatory activity via production of IL-10 and Transforming Growth Factor β. In support of this view, we provided clear-cut evidence that serum levels of IL-10 are increased in HCV+ patients who terminated IFN-α/RIB treatment free of circulating endotoxins (Caradonna et al., 2002). In a recent paper by Finkelman et al. (2006), evidence has been provided that allergen extracts contaminated with the highest content of BG are those endowed with the most successful therapeutic properties in allergic diseases. In particular, an effective allergen immunotherapy is associated with an increase in circulating IL10+ CD4+ CD25+ T cells and in mucosal IFN-γ-secreting T cells (Francis et al., 2003).

Just recently, according to a review by Sutmuller et al. (2006), *Candida albicans* infection leads to an immunosuppressive pathway mediated through TLR-2 and subsequent generation of TREG cells. At the same time, *Candida glucans* via Dectin-1/TLR-2 on MØ mediates an IL-10-dependent immunosuppression. Collectively, these data indicate that BG-mediated anti-inflammatory and immunosuppressive activities could reduce the hepatic damage in HCV infection.

Another important finding is represented by the increase of CD3- CD8+ cells in LPS-/BG+ patients. In general terms, NK cells are able to either exert a direct antimicrobial effect or modulate the innate or adaptive immune response via production of IFN-γ (Sher et al., 1993). At the moment, in our group of patients the expansion of this subset of NK cells is difficult to interpret; however, it may contribute to the observed increase of serum IFN-γ in responders to IFN-γ/RIB therapy (Amati et al., 2002; Caradonna et al., 2002).

With regard to other T cell surface markers in the LPS-/BG+ subset, the increase in CD71+ cells might be related to the expansion of CD25+ cells and NK cells. On the other hand, the increase of CD95+ cells could imply an apoptosis of CD45+RO cells, whose number is decreased, as previously reported in this chapter. These T memory cells likely comprise CD4+ and CD8+ cells specific for viral epitopes and, therefore, actively involved in the hepatolysis. In this respect, evidence has been provided that *in vivo* soluble BG could enhance spontaneous lymphocyte apoptosis, thus contributing to the multiple anti-inflammatory activities of the entire molecule (Abel & Czop, 2002*)*. Conversely, in the LPS+/BG+ subset, a positive correlation was found with CD3+ and CD4+ cells, thus suggesting a continuous proliferation of T effector cells capable of maintaining the inflammatory status.

Quite interestingly, in mice BG abrogate induction of endotoxin tolerance leads to an increased expression of IFN-γ in response to IL-12 and IL-18 (Sherwood et al., 2001). The ability of BG to augment the expression of IFN-γ in LPS-tolerant mice suggests their potential use in the recovery of trauma and sepsis-induced immunosuppression. Therefore, also in HCV+ patients with endotoxemia, BG could contribute to the host protection by enhancing antimicrobial immunity, also attenuating the noxious effect of LPS. At the same time, BG in the absence of LPS may express with higher potency their beneficial role for the host, thus contributing to HCV eradication.

In summary, the HCV+ host is under multiple antigenic challenges (e.g., bacteria and fungi), and the mutual balance between LPS- and BG-induced regulation of the immune system may have important clinical reflections in terms of response or refractoriness to IFN-α/RIB therapy. This fact may correlate with the hypothesis according to which activation of the innate immune system abrogates APC tolerance in the liver, thus rendering CD8+ cells more efficient in their cytotoxic response (Bowen et al., 2005). On the other hand, Wuensch et al. (2006) have demonstrated that infecting hepatocytes with an adeno-associated virus vector, T cell activation is exclusively intrahepatic and does not lead to liver tolerance. In this case local CD8+ cell activation seems to bypass the need for CD4+ T cell help, thus indicating that the liver immune response and tolerance also depend on the type of antigenic challenge involved.

Conclusively, these findings suggest that BG may represent potential new drugs for mitigating the exaggerated hepatic inflammation induced by LPS or other microbial AGs that have entered the HCV+ host. Therefore, calibration of intrahepatic activation of the immune system in HCV disease seems essential for eradicating the virus on the one hand and for avoiding liver damage on the other hand.

References

Abel, G., Czop, J.K. (1992). Stimulation of human monocyte Beta-Glucan receptor by glucan particles production of TNF-alfa and IL-1 beta. *International Journal of Immunopharmacology*, 14: 1363–1373.

Adachi, Y., Moore, L.E., Bradford, B.V., Gao, W., Bojes, H.K., Thurman, R.G. (1995). Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology,* 108: 218–224.

Adachi, Y., Bradford, V., Gao, W., Bojes, H.K., Thurman, R.G. (1994). Inactivation of Kupffer cells prevents early alcohol-induced liver injury. *Hepatology,* 20: 453–460.

Ajuebor, M.N., Carey, J.A., Swain, M.G. (2006). CCR5 in T-cell mediated liver disease: what's going on? *Journal of Immunology*, 177: 2039–2045.

Amati, L., Caradonna, L., Magrone, T., Mastronardi, M.L., Cuppone, R., Cozzolongo, R., Manghisi, O.G., Caccavo, D., Amoroso, A., Jirillo, E. (2002). Modifications of the immune responsiveness in patients with hepatitis C virus infection following treatment with IFN-α/Ribavirin. *Current Pharmaceutical Design,* 8: 981–993.

Amati, L., Cozzolongo, R., Manghisi, O.G., Cuppone, R., Pellegrino, N.M., Caccavo, D., Jirillo, E. (2004). The immune responsiveness in hepatitis C virus infected patients: effects of interferon-alpha/ribavirin combined treatment on the lymphocyte response with special reference to B cells. *Current Pharmaceutical Design,* 10: 2093–2100.

Amati, L., Leogrande, D., Finkelman, M.A., Tamura, H., Jirillo, E. (2005a). Peripheral immunity in patients with hepatitis C virus (HCV) infection: different roles of endotoxins (LPS) and β-glucans. *Journal of Endotoxin Research,* 10: 362 [abstract].

Amati, L., Leogrande, D., Passeri, M.E., Mastronardi, M.L., Passantino, L., Venezia, P., Jirillo, E. (2005). β-glucans: old molecules with newly discovered immunological activities. *Drug Design Reviews-Online,* 2: 251–258.

Bertolino, P., Trescol-Biemont, M.C., Rabourdin-Combe, C. (1998). Hepatocytes induce functional activation of naive CD8+ T lymphocytes but fail to promote survival. *European Journal of Immunology,* 28: 221–236.

Bowen, D.G., Walker, C.M. (2005). Adaptive immune response in acute and chronic hepatitis C virus infection. *Nature,* 436: 946–952.

Bowen, D.G., McCaughan, G.W., Bertolino, P. (2005). Intrahepatic immunity: a tale of two sites? *Trends in Immunology,* 26: 512–517.

Brown, G.D., Taylor, P.R., Reid, D.M., Willment, J.A., Williams, D.L., Martinez-Pomares, L., Wong, S.Y.C., Gordon, S. (2002). Dectin-1 is a major β-glucan receptor on macrophages. *The Journal of Experimental Medicine,* 196: 407–412.

Burgio, V.L., Ballardini, G., Artini, M., Caratozzolo, M., Bianchi, F.B., Levriero, M. (1998). Expression of co-stimulatory molecules by Kupffer cells in chronic hepatitis of hepatitis C virus etiology. *Hepatology,* 27: 1600–1606.

Caccavo, D., Afeltra, A., Pece, S., Giuliani, G., Freudenberg, M., Galanos, C., Jirillo, E. (1999). Lactoferrin-lipid A-lipopolysaccharide interaction: inhibition by anti-human lactoferrin monoclonal antibody AMG 10.14. *Infection and Immunity*, 67: 4668–4672.

Caccavo, D., Pellegrino, N.M., Altamura, M., Rigon, A., Amati, L., Amoroso, A., Jirillo, E. (2002). Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. *Journal of Endotoxin Research,* 8: 403–417.

Cacciarelli, T.V., Martinez, O.M., Gish, R.G., Villanueva, J.C., Krams, S.M. (1996). Immunoregolatory cytokines in chronic hepatitis C virus infection: pre- and posttreatment with interferon alpha. *Hepatology,* 24: 6–9.

Caradonna, L., Mastronardi, M.L., Magrone, T., Cozzolongo, R., Cuppone, R., Manghisi, O.G., Caccavo, D., Pellegrino, N.M., Amoroso, A., Jirillo, E., Amati, L. (2002). Biological and clinical significance of endotoxemia in the corse of hepatitis C virus infection. *Current Pharmaceutical Design,* 8: 995–1005.

Chan, C.C., Hwang, S.J., Lee, F.Y., Wang, S.S., Chang, F.Y., Li, C.P., Chu, J.C., Lu, R.H., Lee, S.D. (1997). Prognostic value of plasma endotoxin levels in patients with cirrhosis. *Scandinavian Journal of Gastroenterology,* 32: 942–946.

Chang, K.M. (1998). The mechanisms of chronicity in hepatitis C virus infection. *Gastroenterology,* 115: 1015–1018.

Conlan, J.W. (1997). Critical roles of neutrophils in host defense against experimental systemic infections of mice by *Listeria monocytogens*, *Salmonella typhimurium* and *Yersinia enterocolitica* . *Infection and Immunity,* 65: 630–635.

Decreus, A., Abe, M., Lau, A.H., Hackstein, H., Raimondi, G., Thomson, A.W. (2005). Low TLR4 expression by liver dendritic cells correlates with reduced capacity to activate allogeneic T cells in response to endotoxin. *Journal of Immunology,* 174: 2037–2045.

Diehl, A.M. (1999). Cytokine and the molecular mechanisms of alcoholic liver disease. *Alcoholic Clinical Experimental Research,* 23: 1419–1424.

Engstad, C.S., Engstad, R.E., Olsen, J.O., Osterud, B. (2002). The effect of soluble beta-(1,3)-glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood. *International Immunopharmacology,* 11: 1585–1597.

Enomoto, N., Ikejima, K., Bradford, B.V., Rivera, C., Kono, H., Brenner, D.A., Thurman, R.G. (1998). Alcohol causes both tolerance and sensitization of rat Kupffer cells via mechanism dependent on endotoxin. *Gastroenterology,* 115: 443–451.

Fax, E.S., Thomas, P., Broitmans, A. (1999). Clearance of gut-derived endotoxins by the liver. Release and modifications of ^{3}H, ^{14}C-lipopolysaccharide by isolated Kupffer cells. *Gastroenterology,* 96: 456–461.

Fearns, C., Kravchenko, V.V., Ulevitch, R.J, Loskutoff, D.J. (1995). Murine CD14 gene expression in vivo: extramyeloid synthesis and regulation by lipopolysaccharides. *Journal of Experimental Medicine,* 181: 857–866.

Finkelman, M.A., Lempitski, S.J., Slater, J.E. (2006). B-glucans in standardized allergen extracts. *Journal of Endotoxin Research,* 12: 241–245.

Francis, J.N., Till, S.J., Durham, S.R. (2003). Induction of IL-10+ CD4+ CD25+ T cells by grass pollen immunotherapy. *Journal of Allergy and Clinical Immunology,* 111: 1255–1261.

Fraser, D.A., Bohlson, S.S., Jasinskiene, N., Rawal, N., Palmarini, G., Ruiz, S., Rochford, R., Tenner, A.J. (2006). C1q and MBL, components of the innate immune system, influence monocyte cytokine expression. *Journal of Leukocyte Biology,* 80: 107–116.

Galanos, C., Freudenberg, M.A., Reutter, W. (1979). Galactosamine-induced sensitization to the lethal effects of endotoxin. *Proceedings National Academy of Sciences USA,* 76: 5939–5943.

Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S., Underhill, D.M. (2003). Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *Journal of Experimental Medicine,* 197: 1107–1117.

Goddard, S., Youster, J., Morgan, E., Adams, D.H. (2004). Interleukin-10 secretion differentiates dendritic cells from human liver and skin. *American Journal of Pathology,* 164: 511–519.

Godfrey, D.I., Kronenberg, M. (2004). Going both ways: immune regulation via CD1d-dependent NKT cells. *Journal of Clinical Investigation,* 114: 1379–1388.

Gregory, S.H., Sagnimeni, A.J., Wing, E.J. (1996). Bacteria in the blood stream are trapped in the liver and killed by immigrating neutrophils. *Journal of Immunology,* 157: 2514–2520.

Gregory, S.H., Cousens, L.P., Van Rooijen, N., Dopp, E.A., Carlos, T.M., Wing, E.J. (2002). Complementary adhesion molecules promote neutrophil-Kupffer cell interaction and the elimination of bacteria taken up by the liver. *Journal of Immunology,* 168: 308–315.

Harcourt, G.C., Lucas, M., Sheridan, I., Barnes, E., Phillips, R., Klenerman, P. (2004). Longitudinal mapping of protective CD4+ T cell responses against HCV: analysis of fluctuating dominant and subdominant HLA-DR11 restricted epitopes. *Journal of Viral Hepatitis,* 11: 324–331.

Hoffman, O.A., Olson, E.J., Limper, A.H. (1993). Fungal beta-glucans modulate macrophage release of tumor necrosis factor-alpha in response to bacterial lipopolysaccharide. *Immunology Letters,* 37(1): 19–25.

Iimuro, Y., Gallucci, R.M., Luster, M.I., Kono, H., Thurman, R.G. (1997). Antibodies to tumor necrosis factor-alpha attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat. *Hepatology,* 26: 1530–1537.

Jabbari, A., Harty, J.T. (2006). The generation and modulation of antigen-specific memory CD8 T cell responses. *Journal of Leukocyte Biology,* 80: 16–23.

Jellison, E.R., Kim, S.K., Welsh, R.M. (2005). Cutting edge: MHC class II-restricted killing in vivo during viral infection. *Journal of Immunology,* 174: 614–618.

Jirillo, E., Greco, B., Caradonna, L., Satalino, R., Pugliese, V., Cozzolongo, R., Cuppone, R., Manghisi, O.G. (1995). Evaluation of cellular immune responses and soluble mediators in patients with chronic hepatitis C virus (cHCV) infection. *Immunopharmacology and Immunotoxicology,* 17: 347–364.

Jirillo, E., Greco, B., Caradonna, L., Satalino, R., Amati, L., Cozzolongo, R., Cuppone, R., Manghisi, O.G. (1996). Immunological effects following administration of interferon-α in patients with chronic hepatitis C virus (cHCV) infection. *Immunopharmacology and Immunotoxicology,* 18: 355–374.

Jirillo, E., Amati, L., Caradonna, L., Greco, B., Cozzolongo, R., Cuppone, R., Piazzolla, G., Caccavo, D., Antonaci, S., Manghisi, O.G. (1998). Soluble (s) CD14 and plasmatic lipopolysaccharides (LPS) in patients with chronic hepatitis C before and after treatment with interferon (IFN)-α. *Immunopharmacology and Immunotoxicology,* 20: 1–14.

Jirillo, E., Pellegrino, N.M., Piazzolla, G., Caccavo, D., Antonaci, S. (2000). Hepatitis C virus infection: immune responsiveness and interferon-α treatment. *Current Pharmaceutical Design,* 6: 169–180.

Jirillo, E., Caccavo, D., Magrone, T., Piccigallo, E., Amati, L., Lembo, A., Kalis, C., Gumenscheimer, M. (2002). The role of the liver in the response to LPS: experimental and clinical findings. *Journal of Endotoxin Research,* 8: 319–327.

Kataoka, K., Muta, T., Yamazaki, S., Takeshige, K. (2002). Activation of macrophages by linear (1-right-arrow 3)-beta D-glucans. Implications for recognition of fungi by innate immunity. *Journal of Biological Chemistry,* 277: 36825–36831.

Knolle, P.A., Limmer, A. (2001). Neighborhood politics: the immunoregolatory function of organ-resident liver endothelial cells. *Trends in Immunology,* 22: 432–437.

Kondo, T., Suda, T. Fukuyama, H., Adachi, M., Nagata, S. (1997). Essential roles of the Fas ligand in the development of hepatitis. *Nature Medicine,* 3: 409–413.

Kronenberger, B., Herrmann, E., Hofmann, W.P., Wedemeyer, H., Sester, M., Mihm, V., Ghahai, T., Zeuzem, S., Sarrklin, C. (2006). Dynamics of CD81 expression on lymphocyte subsets during interferon-α-based antiviral treatment of patients with chronic hepatitis C. *Journal of Leukocyte Biology,* 80: 298–308.

Kuratsune, H., Koda, T., Kurahori, T. (1983). The relationship between endotoxin and the phagocytic activity of the reticuloendothelial system. *Hepatogastroenterology,* 30: 79–82.

Le Moine, O., Marchant, A., Degroote, D., Azar, C., Goldman, M., Deviere, J. (1995). Role of defective monocyte interleukin-10 release in tumor necrosis factor overproduction in alcoholic cirrhosis. *Hepatology,* 22: 1436–1439.

Liehr, H., Grun, M., Brunswig, D., Sautter, T. (1976). Endotoxinamie bei leberzirrhose. *Zeitschrift Gastroenterologie,* 14: 14–23.

Li, W., Krishnadas, D.K., Li, J., Tyrrell, D.L.J., Agrawal, B. (2006). Induction of primary human T cell responses against hepatitis C virus-derived antigens NS3 or core by autologous dendritic cells expressing hepatitis C virus antigens: potential for vaccine and immunotherapy. *Journal of Immunology,* 176: 6065–6075.

Li, X.K., Fujino, M., Sugioka, A., Morita, M., Okuyama, T., Guo, L., Funeshima, N., Rimura, H., Enosawa, S., Amemiya, H., Suzuki, S. (2001). Fulminant hepatitis by Fas-ligand expression in MLR-lpr/lpr mice grafted with Fas-positive livers and wild-type mice with Fas-mutant livers. *Transplantation,* 71: 503–507.

Liu, S., Khemlani, L.S., Shapiro, R.A., Johnson, M.L., Liu, K., Geller, D.A., Watkins, S.C., Goyert, S.M., Billiar, T.R. (1998). Expression of CD14 by hepatocytes: upregulation by cytokines during endotoxemia. *Infection and Immunity,* 66: 5089–5098.

MacPhee, P.J., Schmidt, E.E., Groom, A.C. (1992). Evidence for Kupffer cell migration along liver sinusoids from high-resolution in vivo microscopy. *American Journal of Physiology*, 263: G17–G23.

Maitra, S.K., Rachmilewitz, D., Eberle, D., Haplowitz, N. (1981). The hepatocellular uptake and biliary excretion of endotoxin in the rat. *Hepatology,* 8: 1550–1554.

Marr, K.A., Arunmozhi Balajee, S., Hawn, T.R., Ozinsky, A., Pham, U., Akira, S., Aderem, A., Liles, W.C. (2003). Differential role of MyD88 in macrophage-mediated responses to opportunistic fungal pathogens. *Infection and Immunity,* 71: 5280–5286.

Mignon, A., Rouquet, N., Fabre, M., Martin, S., Pages, J.C., Dhainaut, J.E., Kahn, A., Briand, P., Joulin, V. (1999). LPS challenge in D-galactosamine-sensitized mice accounts for caspase-dependent fulminant hepatitis, not for septic shock. *American Journal of Respiratory Critical Care Medicine,* 159: 1308–1315.

Miyake, K. (2006). Roles for accessory molecules in microbial recognition by Toll-like receptors. *Journal of Endotoxin Research,* 12: 195–204.

Nakagawa, Y., Ohno, N., Muras, T. (2003). Suppression by *Candida albicans* beta-glucan of cytokine release from activated human monocyte and from T cells in the presence of monocytes. *Journal of Infectious Disease,* 187: 710–713.

Nanji, A.A., Khettry, V., Sadrzaden, S.M. (1994). *Lactobacillus* feeding reduces endotoxemia and severity of experimental alcoholic liver disease. *Proceedings Society Experimental Biology Medicine,* 205: 243–247.

Napoli, J., Bishop, G.A., McGuinness, P.H., Painter, D.M., McCaughan, G.W. (1996). Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th-1-associated cytokines. *Hepatology,* 24: 759–765.

Neumann-Haefelin, C., Blum, H.E., Chisari, F.U., Thimme, R. (2005). T cell response in hepatitis C virus infection. *Journal of Clinical Virology,* 32: 75–85.

Ni, H.T., Deeths, M.J., Mescher, M.F. (1999). LFA-1-mediated costimulation of CD8+ T cell proliferation requires phosphatidylinositol 3-kinase activity. *Journal of Immunology,* 166: 6523–6529.

Nolan, J.P. (1975). The role of endotoxin in liver injury. *Gastroenterology,* 69: 1345–1356.

Oesterreicher, C., Pfefel, F., Petermann, D., Muller, C. (1995). Increased in vitro production and serum levels of the soluble lipopolysaccharide receptor sCD14 in liver disease. *Journal of Hepatology,* 23: 396–402.

Opal, S.M., Scannon, P.J., Vincent, J.L., White, M., Caroll, S.E., Palardy, J.E., Parejo, N.A., Pribble, J.P., Lemke, J.H. (1999). Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *Journal of Infectious Disease*, 180: 1584–1589.

Poltorak, A., He, X., Smirnova, I., Liu, M.Y., VanHuffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science,* 282: 2085–2088.

Prytz, H., Holtz-Christensen, J., Korner, B., Liehr, H. (1976). Portal venous and systemic endotoxaemia in patients with cirrhosis. *Scandinavian Journal of Gastroenterology,* 11: 857–863.

Rehermann, B., Nascimbeni, M. (2005). Immunology of hepatitis B virus and hepatitis C virus infection. *Nature Review in Immunology,* 5: 215–229.

Sandberg, J.K., Ljunggren, H.-G. (2005). Development and function of CD1d-restricted NKT cells: influence of sphingolipids, SAP and sex. *Trends in Immunology,* 26: 347–350.

Sansonno, D., DeVita, S., Iacobelli, A.R., Cornacchiulo, V., Boiocchi, M., Dammacco, F. (1998). Clonal analysis of intrahepatic B cells from HCV-infected patients with and without mixed cryoglobulinemia. *Journal of Immunology,* 160: 3594–3601.

Sato, T., Asanuma, Y., Tanaka, J., Koyama, K. (1997). Inflammatory cytokine production enhancement in the presence of lipopolysaccharide after hepatic resection in cirrhotic patients. *Therapeutic Apheresis,* 1: 75–78.

Sato, T., Iwabuchi, K., Nagaoka, I., Adachi, Y., Ohno, N., Tamura, H., Seyama, K., Fukuchi, Y., Nakayama, H., Yoshizaki, F., Takamori, K., Ogawa, H. (2006). Induction of human neutrophil chemotaxis by *Candida albicans*-derived β-1,6-long glycoside-chain-branched β-glucan. *Journal of Leukocyte Biology,* 80: 204–211.

Seljelid, R., Figenschau, Y., Bogwald, J., Rasmussen, L.T., Austgulen, R. (1989). Evidence that tumor necrosis induced by aminated beta 1-3 D polyglucose is mediated by a concerted action of local and systemic cytokines. *Scandinavian Journal of Immunology,* 30: 687–694.

Sher, A., Oswald, I.P., Hieny, S., Gazzinelli, R.T. (1993). *Toxoplasma gondii* induces a T-independent IFN-gamma response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-α. *Journal of Immunology,* 150: 3982–3989.

Sherwood, E.R., Varma, T.K., Fram, R.Y., Lin, C.Y., Koutrouvelis, A.P., Toliver-Kinsky, T.E. (2001). Glucan phosphate potentiates endotoxin-induced interferon-gamma expression in immunocompetent mice, but attenuates induction of endotoxin tolerance. *Clinical Science,* 101: 541–550.

Shoukry, N.H., Sidney, J., Sette, A., Walker, C.M. (2004). Conserved hierarchy of helper T cell responses in a chimpanzee during primary and secondary hepatitis C virus infection. *Journal of Immunology,* 172: 483–492.

Snyder, J.T., Alexander-Miller, M.A., Berzofskyl, J.A., Belyakov, I.M. (2003). Molecular mechanism and biological significance of CTL avidity. *Current HIV Research,* 1: 287–294.

Soltys, J., Quinn, M.T. (1999). Modulation of endotoxin-and enterotoxin-induced cytokine release by *in vivo* treatment with β-(1,6)-branched β-(1,3)-glucan. *Infection and Immunity,* 67: 244–252.

Sutmuller, R.P.M., Morgan, M.E., Netea, M.G., Graver. O, Adema, G.J. (2006). Toll-like receptors on regulatory T cells: expanding immune regulation. *Trends in Immunology,* 27: 387–393.

Tannahil, C.L., Fukuzuka, K., Marum, T., Abouhamze, Z., MacKay, S.L., Copeland, E.M. III, Moldawer, L.L. (1999). Discordant tumor necrosis factor-alpha superfamily gene expression in bacterial peritonitis and endotoxemic shock. *Surgery,* 126*:* 349–357.

Tian, Z., Shen, X., Feng, H., Gao, B. (2000). IL-1 beta attenuates IFN-alpha/beta-induced antiviral activity and Stat1 activation in the liver. *Journal of Immunology*, 165: 3959–3965.

Trobonjaca, Z., Leithauser, F., Moller, P., Schirmbeck, R., Reimann, J. (2001). Activating immunity in the liver. I. Liver dendritic cells (but not hepatocytes) are potent activators of IFN-γ release by liver NKT cells. *Journal of Immunology,* 167: 1413–1422.

Tsutsui, H. Matsui, K. Okamura, H., Nakanishi, K. (2000). Pathophysiological roles of interleukin-18 in inflammatory liver diseases. *Immunological Reviews,* 174: 192–209.

Uhrig, A., Banafsche, R., Kremer, M., Hegenbarth, S., Hamann, A., Neurath, M., Gerken, G., Limmer, A., Knolle, P.A. (2005). Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. *Journal of Leukocyte Biology,* 77: 626–633.

Underhill, D.M., Olinsky, A. (2002). Toll-like receptors: key mediators of microbe detection. *Current Opinion in Immunology,* 14: 103–110.

Urbani, S., Amadei, B., Cariani, E., Fisicaro, P., Orlandini, A., Missale, G., Ferrari, C. (2005). The impairment of CD8 responses limits the selection of escape mutations in acute hepatitis C virus infection. *Journal of Immunology,* 175: 7519–7529.

Van Andel, R.A., Hook, R.R., Jr., Franklin, C.L., Besch-Williford, C.L., Van Roijen, N., Riley, L.K. (1997). Effects of neutrophil, natural killer cell, and macrophage depletion on murine *Clostridium piliforme* infection. *Infection and Immunity,* 65: 2725–2731.

Van Molle, W., Denecker, G., Rodriguez, I., Brouckaert, P., Vandenabeele, P., Libert, C. (1999). Activation of caspases in lethal experimental hepatitis and prevention by acute phase proteins. *Journal of Immunology,* 163: 5235–5241.

Vento, S. (2000). Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *Journal of Viral Hepatitis,* 7: 7–8.

Verdrengh, M., Tarkowsky, A. (1997). Role of neutrophils in experimental septicemia and septic arthritis induced by *Staphylococcus aureus*. *Infection and Immunity,* 65: 2517–2521.

Vodovotz, Y., Liu, S., McCloskey, C., Shapiro, R., Green, A., Billiar, T.R. (2001). The hepatocyte as a microbial product- responsive cell. *Journal of Endotoxin Research,* 7: 365–373.

Weunsch, S.A., Pierce, R.H., Crispe, N.I. (2006). Local intrahepatic CD8+ T cell activation by a non-self-antigen results in full functional differentiation. *Journal of Immunology,* 177: 1689–1697.

Williams, D.L., Ha, T., Li, C., Kalbfleish, J.A., Laffan, J.J., Ferguson, D.A. (1999). Inhibiting early activation of tissue nuclear factor-Kappa B and interleukin-6. (1→3)-Beta-D-Glucan increases long-term survival in polymicrobial sepsis. *Surgery,* 126: 54–65.

Williams, D.L., Ha, T., Li, C., Laffan, J.J., Kalbfleish, J.A., Browder, W. (2002). Inhibition of LPS-induced NFKAPPAB activation by a glucan ligand involves down-regulation of IKKBETA kinase activity and altered phosphorylation and degradation of IKKABALPHA. *Shock,* 13: 446–452.

Williams, D.L.A., Mueller, A., Browder, W. (1998). Glucan-based macrophage stimulators: a review of their anti-infective potential. *Clinical Immunotherapy,* 5: 392–399.

Williams, R.C., Jr., Malone, C.C., Kao, K.J. (1994). IgM rheumatoid factors react with human class I HLA molecules. *Journal of Immunology,* 156: 1684–1694.

Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J., Mathison, J.C. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science,* 249: 1431–1433.

Yin, M., Bradford, B.V., Wheeler, M.D., Uesugi, T., Froh, M., Goyert, S.M., Thurman, R.G. (2001). Reduced early alcohol-induced liver injury in CD14-deficient mice. *Journal of Immunology,* 166: 4737–4742.