

# Obstructive jaundice causes reduced expression of polymorphonuclear leucocyte adhesion molecules and a depressed response to bacterial wall products in vitro

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## Abstract

**Background**—Obstructive jaundice is associated with an increased incidence of infection and endotoxaemia, which may result from impaired host immunity. Neutrophil adhesion to vascular endothelium is a key part of the inflammatory response.

**Aims**—To investigate neutrophil adhesion molecule expression and activation in obstructive jaundice.

**Patients**—Nine adult patients with obstructive jaundice and 11 control subjects.

**Methods**—The expression of the neutrophil adhesion receptors L-selectin, CD11a, CD11b, CD11c, and CD15 was determined using flow cytometry. CD11b expression in response to stimulation with fMLP and endotoxin was measured.

**Results**—The basal expression of L-selectin, CD11a, and CD15 was significantly decreased in jaundiced patients ( $p < 0.05$ ) and the expression of CD11b in response to stimulation with fMLP and endotoxin was significantly impaired in the jaundiced group. Endotoxin stimulation without plasma did not reverse the impaired response showing that it is not caused by endotoxin inactivation by plasma proteins.

**Conclusions**—Neutrophils from patients with obstructive jaundice show decreased adhesion receptor expression and an impaired response to stimulation with bacterial products. This cellular dysfunction may be responsible for the high incidence of septic complications in these patients.

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associated with an increased incidence of bacterial infection.

The  $\beta$ -2 integrins CD11b and CD11a are responsible for firm adhesion, which is an essential prerequisite to migration out of the circulation. CD11b expression is increased by neutrophil stimulation and is a marker of neutrophil activation. The in vivo response to bacterial invasion is mediated by cytokines and the direct stimulation of neutrophils by bacterial wall products such as lipopolysaccharide (LPS) and fMLP, which will increase neutrophil CD11b expression. We have therefore studied the expression of neutrophil adhesion receptors from patients with jaundice and examined the response of CD11b to neutrophil stimulation by LPS and fMLP.

## Methods

### Subjects

Nine patients (four female) with obstructive jaundice were studied. Their mean age was 64 years (range 46-83), eight had malignant biliary obstruction and one had gall stones. Six had malignancy of the pancreas or biliary tree and two had obstruction due to nodes at the porta hepatis from metastatic colon carcinoma. They were compared with 11 control patients (five female) with benign, non-inflammatory disease and a mean age of 53 years (range 26-78). The mean bilirubin concentration in the jaundiced group was 258  $\mu\text{mol/l}$  (range 84-597) and the mean duration of jaundice was four weeks (range 1-11). There was no evidence of clinical infection at the time of the study nor had there been any clinical episodes of infection during the course of their illness. All patients were studied before invasive investigations or surgery was carried out.

### Basal adhesion molecule expression

Venous blood was taken into glass tubes with sodium heparin (10 units/ml) and analysed immediately. Aliquots of whole blood (50  $\mu\text{l}$ ) were incubated on ice with fluorescein conjugated monoclonal antibodies to L-selectin (Leu-8, Becton-Dickinson UK, Oxford, UK), CD11a (MHM 24, Dako, High Wycombe, UK), CD11b (clone 44, Serotec, Oxford, UK), CD11c (KB90, Dako), CD15 core protein (C3D-1, Dako), and control fluorescein conjugated mouse IgG<sub>1</sub> (DAK-GO1, Dako), for 30 minutes. Lysing fluid (Becton-

Infection causes considerable perioperative morbidity in patients with obstructive jaundice.<sup>1,2</sup> Bacterial colonisation of the biliary tree is common<sup>3</sup> but changes in immune response may also be important. Kupffer cell function<sup>4</sup> and delayed hypersensitivity<sup>5</sup> are impaired in obstructive jaundice but human neutrophil function has not been investigated and animal studies have conflicting results.<sup>6,7</sup> Neutrophil adhesion to the vascular endothelium is essential for subsequent transmigratio<sup>8,9</sup> and congenitally impaired adhesion is

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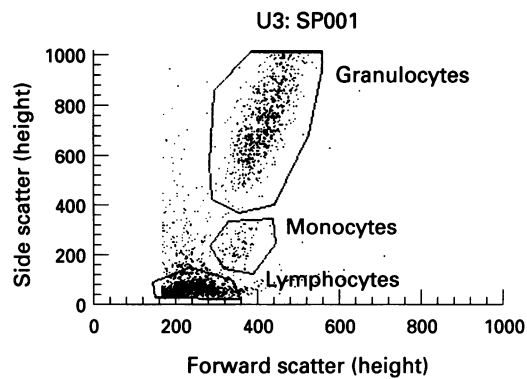
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Figure 1: Neutrophils (granulocytes) identified by their characteristic properties of forward and side scatter by flow cytometry.



Dickinson) was then added and the tubes were incubated at room temperature for 10 minutes. They were then washed in phosphate buffered saline (PBS) containing azide (0.05%) and resuspended in PBS-formaldehyde (1%). Median channel fluorescence of the neutrophil population was determined by flow cytometry (FACSan, Becton-Dickinson, San Jose, USA). The neutrophil population was identified by their properties of forward and side scatter (Fig 1).

#### CD11b expression in response to stimulation

The expression of CD11b in response to stimulation with fMLP (Sigma, Poole, UK) and LPS (*Escherichia coli* O26:B6, Sigma) was determined as follows. Whole blood (0.5 ml) was incubated in endotoxin free tubes at 37°C with  $10^{-6}$ M and  $10^{-8}$ M fMLP or 100 ng/ml LPS. At various time points, 50  $\mu$ l aliquots were added to tubes containing fluorescein conjugated anti-CD11b and incubated as above. Aliquots were taken at 0, 5, 15, and 30 minutes with fMLP, and at 0, 5, 15, 30, 45, and 60 minutes with LPS.

The effect of plasma proteins on the neutrophil response to LPS was investigated by spinning a sample of the heparinised whole blood for five minutes, removing the plasma, and resuspending the cells in an equivalent volume of Dulbecco's PBS-bovine serum albumin 2%, containing calcium (0.9 mmol/l) and magnesium (0.9 mmol/l). The resuspended plasma free cells were then stimulated with LPS and CD11b expression determined at the same time points. Control samples were examined for CD11b expression at all time points after stimulation to examine the effects of rewarming and incubation on the neutrophils.

#### Statistical analysis

Statistical analysis was performed using the Mann-Whitney U test for unpaired comparison of non-parametric data and the Wilcoxon test for paired samples. The hospital clinical research (ethics) committee approved the study and all patients gave informed consent.

#### Results

##### Basal adhesion molecule expression

L-selectin expression was significantly less in

the jaundiced group (median channel fluorescence (MCF) 18.4, control 42.0,  $p < 0.05$ ). The unstimulated expression of CD11a and CD15 was also significantly depressed in the jaundiced group (MCF CD11a 38.6, control 62.7, CD15 106, control 256,  $p < 0.05$ ). There was no difference in the unstimulated expression of CD11b (MCF 10.2, control 11.6) or CD11c (MCF 6.4, control 7.8).

#### CD11b expression in response to stimulation

CD11b expression in response to stimulation with fMLP and LPS was significantly less in the jaundiced group. Stimulation with  $10^{-8}$ M fMLP (Fig 2) produced significantly less CD11b expression at 15 and 30 minutes after stimulation with  $10^{-6}$ M fMLP the expression was significantly less at all time points (Fig 2). The response to LPS took longer to occur but at 45 and 60 minutes the response was also significantly less in the jaundiced group ( $p < 0.05$ ) (Fig 3). Single populations of neutrophils were seen at all time points (Fig 4).

The removal of plasma had no effect on CD11b expression in the jaundiced group, but the basal expression in the control group was significantly increased in the plasma free cells and this increase was significantly greater at all time points except 45 minutes. Stimulation with LPS without plasma did not affect the relation between the two groups. The expression of CD11b was significantly less at 45 and 60 minutes in the jaundiced group (Fig 3).

#### Discussion

The high morbidity associated with surgery and endoscopic cannulation of the biliary tree in obstructive jaundice remains a significant problem. Awareness of the risk of renal impairment related to perioperative systemic

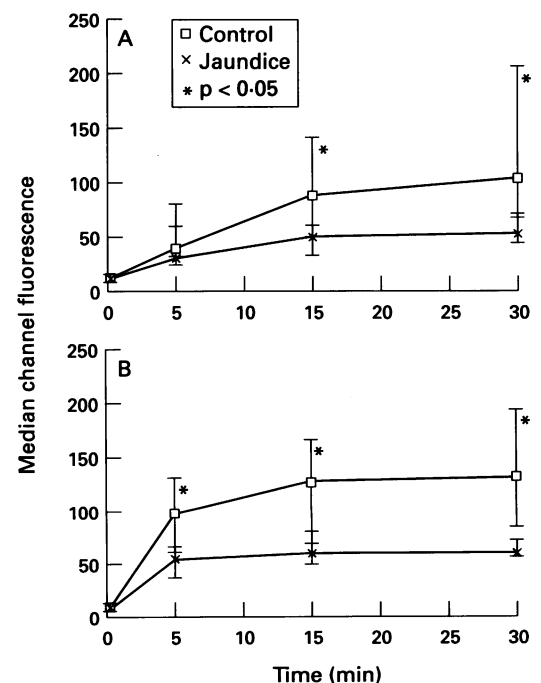


Figure 2: Neutrophil CD11b expression in response to stimulation with (A) fMLP  $10^{-8}$ M and (B) fMLP  $10^{-6}$ M. Medians and interquartile ranges shown.

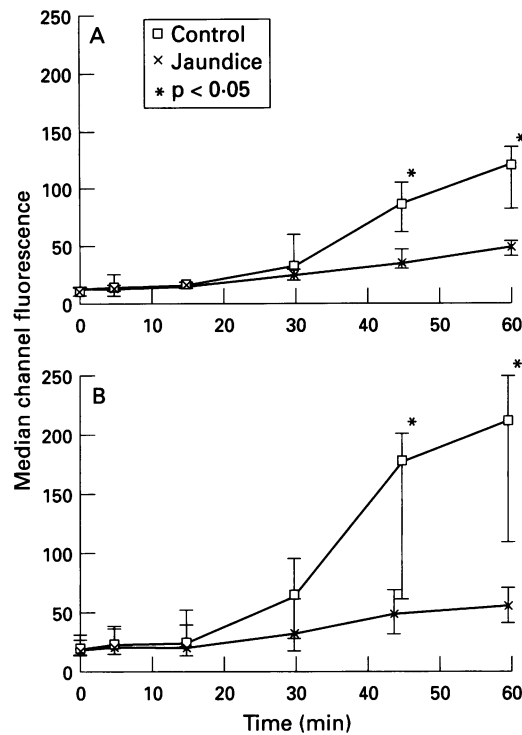


Figure 3: Neutrophil CD11b expression in response to lipopolysaccharide 100 ng/ml (A) with and (B) without plasma. Medians and interquartile ranges shown.

endotoxaemia<sup>10</sup> and the adoption of prophylactic measures such as oral bile salts or pre-operative biliary drainage has limited the incidence of this complication but infection remains common. Cannulation of an obstructed biliary tree results in cholangitis in up to 25% of cases.<sup>11</sup> The reason for the increased incidence of septic complications is unclear.

Neutrophils are a critical component of the response to infection and neutrophil adhesion is essential to allow localisation to the area of bacterial invasion. Adhesion occurs in two steps. The initial margination or 'rolling' of the neutrophil along the vessel wall is mediated by L-selectin and probably CD15 as a ligand for endothelial P-selectin.<sup>12,13</sup> This is followed, if transmigration is to occur, by firmer adhesion mediated by the  $\beta$ -2 integrins, CD11a, CD11b, and CD11c.<sup>14</sup> This study has shown abnormalities in neutrophil function in obstructive jaundice.

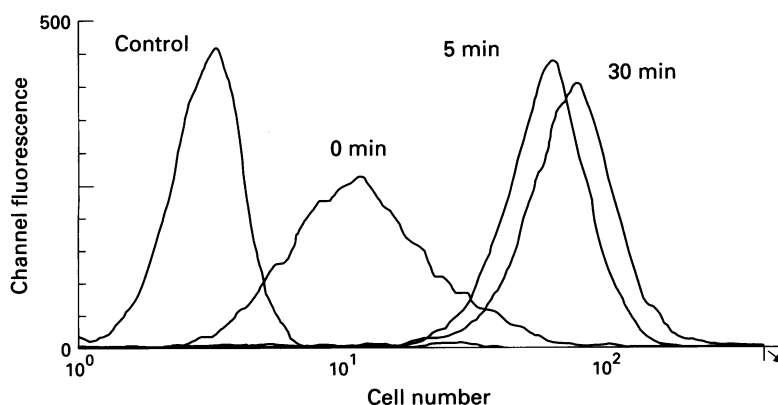


Figure 4: Neutrophil stimulation in response to the entire population. Overlaid histograms of the response to stimulation with fMLP.

The expression of L-selectin, CD11a, and CD15 was reduced in jaundice and thus both stages of the adhesion process may be affected. The antibody used against CD15 is for the core protein and is not specific for the sialylated form, which is more important in rolling. The importance of the actual level of expression of CD15 in rolling is not known. It is not possible therefore to draw definite implications on the functional effects of these changes. Bemelmans *et al*<sup>15</sup> showed the presence of a persistent inflammatory response to obstructive jaundice evidenced by increased values of interleukin 6 and tumour necrosis factor  $\alpha$ . In vivo neutrophil stimulation might lead to increased CD11b expression and shedding of L-selectin. Though L-selectin values were reduced, the unstimulated expression of CD11b was unchanged. If an inflammatory response did lead to the activation of circulating neutrophils these may rapidly adhere and the cells obtained by venepuncture could represent a less active subset. None the less these results do not support the presence of activated neutrophils.

Human studies have shown impaired delayed hypersensitivity<sup>5</sup> and Kupffer cell phagocytosis.<sup>4</sup> Animal studies have shown impaired hepatic clearance of bacteria<sup>16</sup> and candida<sup>17</sup> and impaired cellular immunity in rats.<sup>18</sup> Jaundice has been shown in two studies to increase macrophage function in rats<sup>18</sup> and mice<sup>15</sup> but a third study showed decreased macrophage tumour necrosis factor  $\alpha$ , superoxide and nitric oxide production in response to endotoxin.<sup>19</sup> The function of neutrophils, which with macrophages are the most important components of host defence against bacterial invasion, has not been extensively studied. Two studies in rats have produced conflicting results, one showed impaired peritoneal neutrophil chemotaxis in response to fMLP<sup>6</sup> while the other showed normal chemotaxis but impaired phagocytic response.<sup>7</sup>

CD11b is a marker of neutrophil response to stimulation. It is stored intracellularly in granules that rapidly fuse with the cell membrane upon stimulation resulting in a rapid upregulation of receptor numbers, which is detectable by five minutes.<sup>20</sup> CD11b expression in response to stimulation is a sensitive marker of neutrophil activation. This study has clearly shown an impaired response to stimulation with bacterial products. LPS and fMLP activate neutrophils through different pathways. fMLP stimulates neutrophils directly by specific receptors on the surface membrane<sup>21</sup> while the response to LPS is dependent on the circulating concentrations of plasma proteins. Bacterial permeability increasing protein, produced by neutrophils, inactivates LPS.<sup>22</sup> Conversely the hepatic acute phase protein lipopolysaccharide binding protein greatly increases its activity.<sup>23</sup> The response also depends upon the expression of its receptor CD14.<sup>24</sup> This is shed on activation and is detectable in plasma<sup>25</sup> where it may continue to bind LPS. Thus the cellular response to LPS is dependent upon a complex interaction of various circulating factors and cell receptor

levels. The effects of plasma proteins were minimised by using a large dose of LPS at which the presence of protein ligands is less important,<sup>26</sup> and part of the study was performed using plasma free cells. Unseparated cells were used to avoid the stimulatory effects that neutrophil isolation has on the expression of neutrophil adhesion receptors.<sup>27</sup> An indirect stimulatory effect of LPS by monocyte cytokine secretion is possible in this model, but the effect of fMLP on the neutrophil is direct. This suggests decreased response at the level of the neutrophil itself.

The impaired neutrophil response to endotoxin agrees with the finding of impaired macrophage function.<sup>19</sup> Jaundiced patients undergo episodes of endotoxaemia, which may result in subsequently impaired response to further stimulation.<sup>28</sup> They have also been shown to have increased translocation of bacteria into mesenteric lymph nodes.<sup>19</sup> This may be because of the absence of bile acids from the gut. A persistent inflammatory response as seen in mice<sup>15</sup> could lead to increased interleukin 10 production, which would suppress both neutrophil and macrophage function and may be important. Another explanation for impaired function is the high incidence of neoplastic causes for jaundice. Most patients had disease limited to the hepatobiliary region but none the less malignancy and its associated catabolic state may well have significant effects on neutrophil function.

Removal of plasma had the effect of increasing neutrophil response to stimulation by LPS in the control group. The lack of effect in the jaundiced group may be further indication of hyporesponsiveness in jaundice but could also indicate suppression of control neutrophil function by a serum factor. Neutrophils are very sensitive to isolation procedures and it is possible that stimulation and priming occurs during centrifugation and resuspension. Removal of LPS binding proteins and soluble CD14 may also be important.

In summary this study has shown a defect in neutrophil function in obstructive jaundice in humans. These findings may explain the high incidence of localised sepsis in these patients. The impaired response to bacterial products, however, may also result in a relative resistance to the development of systemic sepsis with organ failure. It could therefore be of some benefit to a patient with jaundice.

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