charge transfer has been discussed in terms of Gerischer's model and modified by inclusion of surface states in the band-gap region. In the bulk of the Nf/MPC film, nitrite ion plays an active role as a dopant of the *p*-type MPC films. For MPC films, in the intrinsic case, the width of the band gap in a slip-stack orientation of adjacent molecules is around 2.0 eV (ref. 10). Light irradiation results in a $S_0 \rightarrow S_1$ transition, i.e. the formation of MPC* located about 0.2 eV below the conduction band-edge. It is assumed that in a semiconductor, the photoexcited electrons in the more negative conduction band have a greater ability to reduce the nitrite ion (Scheme 1). TEA acts as a hole scavenger. It is also reasonable to assume that the field in the microenvironmental site of the Nf membrane[13] will accelerate the multi-electron nitrite-reduction process.

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Study on pinocytosis by monocytes from visceral leishmaniasis patients

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In the present study, we report that monocytes obtained from active visceral leishmaniasis (VL) patients show low rate of pinocytosis compared to that of healthy controls. Several studies have suggested that any change in pinocytic activity reflects changes in the functional state of the cell. In the present study we have evaluated pinocytosis by monocytes obtained from patients with VL. A reduced pinocytic activity by monocytes from active VL patients was observed in comparison to healthy controls. Our study suggests that reduced pinocytic rate by monocytes in active VL infection reflects down-regulation of macrophage activation or functional state.

LEISHMANIA parasites infect exclusively macrophages of the mammalian hosts and subsequently live in the phagolysosomes of these cells[1]. In view of such myriad immune evasion mechanisms of parasites, one might wonder how it is possible for host immunity to contain and resolve parasitic infections. Resolution of infection requires that the host generate an immune response with a cellular (T-cell) component such that activation of macrophages occur. Analysis of the development of activation was facilitated when the operationally defined stage of activation was characterized using a library of objective markers[2].

Among the most characteristic properties of monocytes and macrophages is the capacity of these cells to take up large volumes by fluid-phase pinocytosis, and to ingest microbes and other particles by phagocytosis. Phagocytosis and pinocytosis require similar changes in the actin cytoskeleton, and both processes are sensitive to certain dominant negative mutants of the small ras-like GTPase of the Rho family[3–5], and are blocked by inhibitor phagosphoinositide-30-kinase activity. Fluid-phase pinocytosis by macrophage is a constitutive process[6].

Our earlier studies on state of macrophage activation in active visceral leishmaniasis (VL) patients showed reduced arginase, 5′-nucleotidase, lysozyme, NADH-oxidase, NADPH-oxidase and myeloperoxidase levels (unpublished observations) in monocytes. This prompted us to study pinocytic activity of monocytes from active VL patients and healthy controls. Though the importance of internalization is still unclear, immunological experi-

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ments have suggested that any change in pinocytic activity reflects changes in the functional state of the cell.

All the reagents were prepared in autoclaved quartz distilled water. Ficoll-hypaque (Lymphoprep) was obtained from Nycomed Pharma, Norway. RPMI 1640, HBSS, foetal calf serum (FCS) and other reagents were purchased from Sigma Chemical Co, MO, USA. Culture plates were purchased from A/S Nunc, Denmark.

Blood samples of active patients ($n = 15$) and healthy controls ($n = 9$) were collected from Kala-azar Medical Research Centre, Sir Sundar Lal Hospital, Institute of Medical Sciences, Banaras Hindu University Varanasi and its field side at Muzaffarpur, Bihar.

Human peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood (10 ml) by Ficoll-hypaque density gradient method[7], as described elsewhere. PBMCs were resuspended in RPMI-1640 supplemented with 10% heat-inactivated FCS, gentamycin (20 µg/ml), streptomycin (100 µg/ml) and penicillin (100 U/ml). Monocytes (2×10^6 cells/ml) were obtained by adherence for 2 h at 37°C in tissue-culture plates in a CO_2 incubator (5% CO_2, 95% humidity). Viability of the cells was checked before and after adherence by trypan blue exclusion test. Ninety-nine and 98% cells excluded dye before and after adherence respectively.

Monocytes (2×10^5/well) were plated as described above in 24-well plastic tissue culture plates. Monocytes were treated with 50 µg/0.5 ml of horse radish peroxidase (HRP) in complete medium for 1 h. After the exposure of monocytes to HRP, the cells were washed 5 times in PBS and then lysed in 0.05% Triton X-100 in distilled water. About 2.5 ml of the assay buffer (consisting of 6.0 ml of 0.1 M sodium phosphate buffer pH 5.0, 0.06 ml of 0.3% H_2O_2, 0.05 ml of 10 mg/ml O-Dianisidine) was added to 0.4 ml of cell lysate, and the resulting reaction mixture was kept at 37°C for 1 h (refs 8 and 9). Optical density of supernatants was taken at 460 nm. Following Williams *et al.*[9], a rate of uptake so expressed is termed as endocytic index. The units of endocytic index are µl/10^6 cells. Rate of pinocytosis was expressed as nanolitres/hour of reaction[10].

The clinical and laboratory features of the 15 patients on admission and post-treatment are summarized in Table 1. All the cases had a history of fever, leucopenia, thrombocytopenia, low hemoglobin and hepatospenomegaly (Table 1). Leukocyte counts ranged between 4000 and 7000 mm^3. None of the cases had lymphoadenopathy. The parasite counts in the splenic aspirate ranged from 2^+ to 5^+ (ref. 11). The response to antileishmanial therapy (sodium antimony gluconate or miltefosine) was prompt, and splenic aspirates of all patients at post-treatment showed no parasites (*L. donovani* bodies), confirming that the patients were parasitologically cured. These patients were subsequently monitored for other haematological tests. It was observed that there was recovery in leukocytes and platelets count, haemoglobin levels and reduction in spleen size (Table 1). These observations demonstrate that successful treatment leads to a recovery of immune response. The mechanism by which this happens has been shown in a mouse model, where antileishmanial therapy has been shown to reduce the overall antigenic loads. The mechanism that permits a Th2–Th1 switch may have its roots at the level of the infected macrophages, in that the destruction of a large number of amastigotes within the macrophages may lead to changes in the function of these cells[12].

Monocytes were isolated from healthy controls and active VL patients and assayed for pinocytosis activity. Monocytes of active VL patients showed significantly low pinocytic rate compared to healthy controls (Table 2). Increased but not significant pinocytic rate activity was observed in monocytes from active VL patients on treatment with lipopolysaccharide (LPS) (10 µg/ml) compared to healthy controls.

Peppelenbosch *et al.*[13] (1999) reported that at lower LPS concentration (0.1 to 10 ng/ml), pinocytosis was diminished in both macrophages and monocytes, whereas in macrophages at high LPS concentration (10 µg/ml), this process was stimulated relative to untreated controls. In monocytes, stimulation of pinocytosis by LPS was not

Table 1. Clinical and laboratory features of patients with visceral leishmaniasis (VL) and controls

	Study entry		
	VL Patients		
	Pre-treatment	Post-treatment	Controls
No. of patients	15	15	9
Age (years)	23.40 ± 3.68	–	27.70 ± 3.60
Duration of illness (months)	4.47 ± 0.40	–	–
Weight (kg)	44.59 ± 5.62	47.30 ± 5.40	59.00 ± 5.67
Karnofsky score*	77.00 ± 1.75	86.00 ± 1.72	100
Spleen size (cm)	8.45 ± 1.47	4.23 ± 1.40	–
Splenic aspirate score#	2.55 ± 0.42	0	–
WBC count (× 10^3/mm^3)	3.68 ± 0.76	4.85 ± 1.20	8.60 ± 1.27
Haemoglobin (g/dl)	9.10 ± 0.60	11.23 ± 0.50	14.30 ± 1.72
Platelet count (× 10^3/mm^3)	0.76 ± 0.24	1.42 ± 0.23	184.00 ± 21.21

*Karnofsky performance scale:

Able to carry on normal activity: No special care is needed.	100	– Normal, no complaints, no evidence of disease
	90	– Able to carry on normal activity, minor signs or symptoms of disease
	80	– Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home, and for most personal needs, a varying amount of assistance is needed.	70	– Care required, unable to carry on normal activity or to do active work.

#Splenic aspirate score: Grading of the parasites (ref. 11)

0	0	parasites/1000 field
1^+	> 1–10	parasites/100 field
2^+	> 1–10	parasites/100 field
3^+	> 1–10	parasites/10 field
4^+	> 1–10	parasites/field
5^+	> 10–100	parasites/field
6^+	> 100	parasites/field

Table 2. Pinocytic rate (nl × 10^3/h in 2 × 10^5 cells) of cultured monocytes from active VL patients and healthy controls

Status	Pinocytic rate (nl × 10^3/h/2 × 10^5/cells)
Acute VL patients (n = 15)	
Medium alone	87.00 × 10^3 ± 3.23
LPS (10 µg/ml)	109.80 × 10^3 ± 2.40
Uninfected (n = 9)	
Medium alone	113.33 × 10^3 ± 2.03*
LPS (10 µg/ml)	115.00 × 10^3 ± 0.428

*Active VL patients vs healthy controls, $P < 0.01$.

observed, and only inhibition of fluid-phase uptake by LPS was noted.

In the present study, we report that monocytes obtained from active VL patients show decreased rate of pinocytosis compared to healthy controls. As reported earlier, VL patients show antigen-specific immunosuppression by delayed type hypersensitivity (DTH) and lymphocyte proliferation tests. However, lymphocyte proliferation was observed in response to phytohemagglutinin (PHA), suggesting that immunosuppression is antigen-specific and not generalized. With LPS there was an increase in pinocytic activity in active VL patients, which was not significant compared to healthy controls. Pinocytosis helps in selective internalization of plasma membrane constituents which ensure that important functional components, such as ($Na^+ + K^+$) ATPases are retained on their surface membrane[14].

It is well accepted that antigen-induced activation of T-lymphocyte is macrophage-dependent. Most of the protein and particulate antigens can bind to the surface of accessory cells to greater or lesser extent. The binding of soluble antigens is directly related to the size of the protein, i.e. larger molecules such as keyhole limpet haemocyanin (KLH) bind in significantly greater quantities than smaller molecules like ovalbumin. Another potential pathway of antigen uptake, i.e. fluid-phase pinocytosis may also serve as a means for the endocytosis of antigens, which results in appropriate antigen processing and presentation. Reduced rate of pinocytosis observed in VL patients could explain one possible mechanism for defective antigen processing and its presentation by parasitized macrophages. As widespread parasitization of the mononuclear phagocyte system by *Leishmania* is especially prominent in the spleen and liver, with massive enlargement of these organs, it is not unusual to observe a low pinocytic rate in active VL patients, possibly impairing the $Na^+ + K^+$ ATPases. Not much literature is available on pinocytic activity of macrophages. The importance of interiorization is still unclear, but immunological experiments have suggested that any change in pinocytic activity reflects changes in the functional state of the cell.

It has been reported that crude lymphokine supernatants derived from stimulated T-lymphocytes contain a number of biological activities which affect the macrophage function and enhance pinocytosis[15], alter the energy metabolism and superoxide formation[16], increase oxidative metabolism and microbicidal properties[17]. According to Kreuter[18], host-cell phagocytosis or phagolysosomes-directed pinocytosis may be used for specific drug targeting against *Leishmania* parasites.

Though this is a study done with small number of VL patients, however, it indicates that reduced pinocytic rate by macrophages in active VL reflects down-regulation of macrophage activation or functional state as a consequence of infection. Pending detailed evaluation of pinocytosis in larger number of VL patients, we believe that this could throw light on *Leishmania* infection and macrophage activation in immunopathogenesis of VL.

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