DENDRITIC LEUCOCYTES AS POSSIBLE CARRIERS OF MURINE *PLASMODIUM* MEROZOITES. PRELIMINARY NOTE

BOULARD Y.*, COQUELIN F.*, MORA SILVERA E.*, RENIA L.**,
GAUTRET P.*, DEHARO E.*, SNOUNOU G.***, VUONG P.N.****, CHABAUD A.* & LANDAU I.*

Summary :

A merozoite of *Plasmodium yoelii nigeriensis* was observed, by transmission electron microscopy, associated with the surface of a dendritic leucocyte, in the cortical zone of a renal lymph node from a heavily infected mouse.

KEY WORDS : merozoite, murine *Plasmodium*, dendritic leucocytes, lymphatic system.

Résumé : POSSIBILITÉ D'UNE PHORÉSIE DES MÉROZOITES DE *PLASMODIUM* PAR LES CELLULES DENDRITIQUES. NOTE PRÉLIMINAIRE.

En microscopie électronique, un mérozoïte de Plasmodium yoelii nigeriensis *a été trouvé associé à la surface d'une cellule dendritique dans la zone corticale d'un ganglion lymphatique rénal d'une souris très infectée.*

MOTS CLÉS : *mérozoïte,* Plasmodium *de rongeurs, cellules dendritiques, système lymphatique.*

Observations with murine malaria parasites (reviewed by Landau and Chabaud, 1994) indicate that some merozoites do not enter the red blood cells immediately after their release from mature blood schizonts. It was hypothetized that they leave the bloodstream and enter the lymphatic system, where they remain for variable lengths of time. The discovery of malaria parasites in the lymphatic circulation (Landau *et al.*, 1995) confirmed this hypothesis. Furthermore, observations by Deharo *et al.*, 1995, showed that variations of the lymphatic flux, such as a post prandial increase, have an impact on the parasitaemia of *Plasmodium yoelii nigeriensis*.

In this report we present an interesting finding of a *P. y. nigeriensis* merozoite associated with the surface of a dendritic leucocyte, observed by transmission electron microscopic examination of the renal lymph node from a heavily infected mouse.

* Laboratoire de Biologie Parasitaire et Chimiothérapie (CNRS ERS 156) et Laboratoire de Protozoologie et Parasitologie comparée (EPHE), Muséum National d'Histoire Naturelle, 61, rue Buffon, 75231 Paris Cedex 05.

** Unité INSERM U313, Hôpital de la Pitié Salpétrière, Bd de l'Hôpital, 75005 Paris.

*** Department of Infection and Tropical Medicine, Imperial College School of Medicine. Lister Unit, Northwick Park Hospital, Harrow Middelsex HAI 3UJ, United Kingdom.

**** Unité d'Anatomie et de Cytologie pathologique, Hôpital Saint Michel, 33, rue Olivier de Serres, 75015 Paris.

MATERIALS AND METHODS

RODENT INFECTION

A male Swiss mouse (478 LN) IOPS-OF1 (Iffa-Credo, France) weighing 20 g was inoculated intraperitoneally with blood from a mouse infected with *P. y. nigeriensis*. Four days post-inoculation, when parasitaemia reached 30 %, the mouse was autopsied and the renal lymph node was processed for electron microscopy studies.

ELECTRON MICROSCOPY

The renal lymphatic node was cut into 1 mm^3 pieces, which were fixed at room temperature for 60 minutes, in a 2.8 % glutaraldehyde solution in a 0.1 M Sörensen phosphate buffer (pH = 7.4), washed twice in the buffer and post-fixed 60 minutes, in a 2 % osmium tetroxide solution in the same buffer. Potassium ferricyanide was added to both fixatives. The samples were then stained for 12 hours in 0.5 % uranyl acetate in distilled water. Following dehydration, the material was embedded in a 1:1 mixture of Araldite-Epon®. Sections were stained with uranyl acetate and lead citrate for examination with a PHILIPS EM 201 at the Centre Interuniversitaire de Microscopie Électronique-Jussieu Paris VI.

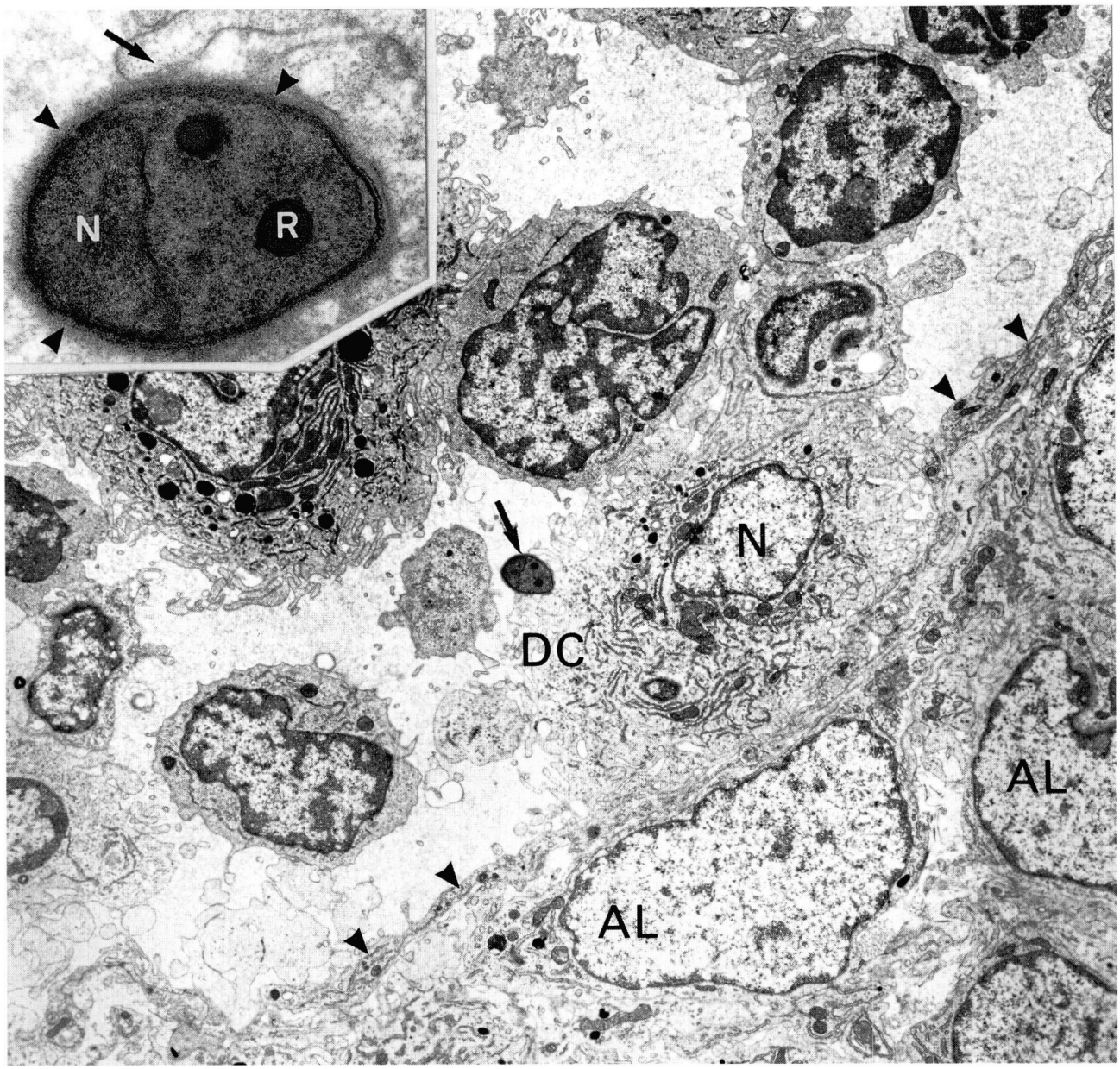

Fig. 1. – Partial vue of the cortical zone of a renal lymph node showing a merozoite (arrow) at the surface of a dendritic cell characterized by two long pseudopodia (arrow-heads) a clear cytoplasm; AL : activated lymphocyte (× 7,000). Insert is a higher magnification of the merozoite showing two rhoptries (R), the nucleus (N), the cell coat around the parasite (arrow-heads) and a microvillus of the dendritic cell (arrow) (× 40,000).

RESULTS

In the cortex of the lymph node, ultrastructural study revealed a malaria parasite adhering to a mononuclear cell (Fig. 1).

The morphology and ultrastructure of the parasite was that of a typical merozoite, *i.e.* a wall with a single plasmalemma surrounding a subpellicular complex made up of two membranes and two microtubules. On one side, a large nucleus without nucleolus, filled about a third of the parasite's volume and, on the opposite side, near the leucocyte, two dense profiles of rhoptries were present. Several small areas of aggregated ribosomes and a few reticulum cisternae were observed inside the cytoplasm. The merozoite was entirely surrounded by a conspicuous cell coat, which was more electron dense than that of the surrounding white cells, and that of merozoites seen in the blood either free or enclosed in mature schizonts.

The nucleus of the cell with which the merozoite was associated, was relatively small with a smooth outline, a thin layer of heterochromatin beneath the nuclear envelop and a clear nucleoplasm. The principal characteristic of the cell was its cytoplasm with many ruffled external protrusions of variable size and length, two of them being particularly long. The Golgi apparatus was not developed but many short lamellae of rough endothelial reticulum were dispersed inside a clear cytoplasm where some mitochondrial profiles and a few small very electron-dense granules were also present. No digestive vacuole was observed. About a third of the surface of the merozoite was in contact with the plasmalemma of the cell, which formed a small cupule at the point of contact with the parasite; a short cytoplasmic digitation of the mononuclear cell was also in close contact with the parasite.

DISCUSSION

In this note, we report the physical interaction of a merozoite with the surface of a mononuclear cell. The cell described above shows many characteristics of dendritic cells: irregular outline with many microvilli and two longer pseudopodia; gathering of the cellular organelles around a central nucleus with a peripheral, clear cytoplasm, devoid of organelles; absence of phagocytosis vacuoles and of digestion residues. Dentritic cells are known to be involved in the initiation of the immune response (Steinman, 1991). In contrast to macrophages, dentritic cells do not possess a pathway for the complete digestion of proteins to amino acids in lysosomes (Steinman and Swanson, 1996); but immature cells (such as Langerhans cells) have the capacity of macropinocytose antigens (Sallusto *et al.*, 1996). This process activates dentritic cells which then migrate to lymph nodes where they initiate an immune response (Steinman and Swanson, 1996).

The fact that we did not observe Birbeck granules and that the rough reticulum is relatively abundant, very dispersed, and does not form lamellar pilings, suggest that this cell has migrated to the lymph nodes and has a mature phenotype. It has also been reported that dendritic cells in the lymph nodes could display particulate antigens at their surface (like HIV particles) (Schmitz *et al.*, 1994). Our observation suggests that the merozoite might be similarly displayed at the surface of the dendritic cell. This is reinforced by the fact that we observed persistence of the coated mantle on the portion of the merozoite in contact with the dendritic cell. The disappearance of the merozoite cell coat in the internalized portion of the parasite during the invasion of the red blood cells is a well-known feature (Bannister *et al.*, 1975) and indicates that, in the present observation, the merozoite had not penetrate inside the cell. No vacuole membrane was observed around the merozoite, further indicating that the merozoite was not endocytosed. The role of the spleen, and especially macrophages it contains, in the destruction of the malaria parasites is relatively well-known. However it appears that, in addition to their destructive capacity, splenic macrophages are also capable of accumulating merozoites which remain apparently intact inside clearly delimited cytoplasmic compartments.

Thus we propose that dendritic cells could transport merozoites from the blood to the lymph node. This property was recently demonstrated for another parasite, *Leishmania major* (Blanck *et al.*, 1993; Moll *et al.*, 1993), which is ingested by epidermal Langerhans cells in the skin and transported to the draining lymph node for presentation to the antigen-specific T cells and initiation of the immune response (Axelrod *et al.*, 1994; Moll *et al.*, 1995).

The existence of trafficking of merozoites of malaria parasites from the bloodstream to the lymphatic compartment, where they could remain viable for lengthy periods, and interact with professional antigen presenting cells, has major implications to our understanding of malaria biology and immunity.

ACKNOWLEDGEMENTS

We are very grateful for the advise and help of Dr. S. Knight, N. N. English, S. S. Patterson and P. P. Konecny from the Antigen Presentation Research Group, Imperial College School of Medicine, Harrow, U.K.

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Reçu le 26 septembre 1996
Accepté le 30 octobre 1996