Journal de la Société de Biologie, 199 (3), 199-206 (2005)

Immunopathological consequences of the loss of engulfment genes: the case of ABCA1

par Georges E. Grau (*) & Giovanna Chimini (#)

Immunopathology Group, Faculté de Médecine, IFR48, 27, boulevard Jean Moulin, F-13385 Marseille Cedex 5, France ; (#) Centre d'Immunologie INSERM CNRS de Marseille Luminy, Case 906, Parc Scientifique de Luminy, 13288, Marseille Cedex 09 ; (*) Université de la Méditerranée, CNRS UMR 6020
Adresse pour correspondance : Giovanna Chimini, Centre d'Immunologie INSERM CNRS de Marseille Luminy, Case 906, Parc Scientifique de Luminy, 13288, Marseille Cedex 09. Tél. : 33 (0)4 91 26 94 04. Fax : 33 (0)4 91 26 94 30. E-mail : chimini@ciml.univ-mrs.fr

Reçu le 15 février 2005

RÉSUMÉ **Conséquences immunopathologiques de la perte de récepteurs pour l'« engulfment » : l'exemple d'ABCA1**

Le programme de la mort cellulaire par apoptose joue un rôle clef dans le maintien de l'homéostasie cellulaire. Une phase initiale, effectrice, conduit à la production de corps apoptotiques et est suivie de près d'une clairance rapide par des macrophages professionnels ou amateurs. De nombreux aspects distinguent ce dernier processus d'« engulfment », ou « engloutissement », de cellules mourantes des formes classiques de phagocytose. Ils concernent toutes les étapes du processus, depuis la reconnaissance de la proie jusqu'au stade final, c'est-à-dire, le « silence immunologique ». L'« engloutissement » de cellules mortes est un processus hautement conservé au cours de l'évolution et a été étudié en parallèle dans deux systèmes, les cellules de mammifères et le nématode *Caenorhabditis elegans*.

ABCA1 et son orthologue CED-7 chez le nématode sont des éléments clés dans l'« engloutissement ». Leur mode d'action est original parmi la myriade de récepteurs pour l'« engloutissement », puisqu'ils agissent comme des transporteurs de lipides. Alors que chez *C. elegans*, la perte de CED-7 a des conséquences phénotypiques exclusivement sur l'« engloutissement », chez la souris, la délétion d'ABCA1 par recombinaison homologue a mis en lumière des manifestations très diverses dans le domaine de la biologie du macrophage.

Parmi celles-ci, nous avons observé des réponses aberrantes des souris ABCA1$^{-/-}$ à l'infection par *Plasmodium berghei* ANKA, particulièrement en ce qui concerne le développement du neuropaludisme, une réaction immunopathologique médiée par les cytokines. Ce syndrome implique les monocytes/macrophages et des taux circulants élevés de microparticules circulantes. Nous avons montré que la perte d'ABCA1 confère une protection complète contre le neuropaludisme et la mortalité qui y est associée. Avec la démonstration de modifications quantitatives et qualitatives des microparticules, cette observation suggère que les microparticules pourraient être impliquées dans la pathogenèse du neuropaludisme. Le transporteur ABCA1 contrôle donc la sensibilité à ce syndrome, offrant de nouvelles possibilités tant sur le plan mécanistique que thérapeutique.

SUMMARY

Programmed cell death plays a crucial role in the maintenance of cell homeostasis. An initial, effector phase leads to the generation of apoptotic corpses and is closely followed by a swift clearance by professional or amateur phagocytes. Several aspects distinguish this latter process of engulfment of dying cells from the classical forms of phagocytosis. They concern all aspects of the process from the recognition of the prey to the final outcome, i.e. immunological silence.

The engulfment of dead cells is a process highly conserved through evolution and it has been studied in parallel in two systems, mammalian cells and the nematode *C. elegans*.

ABCA1 and its ortholog CED-7 in the nematode are key players of engulfment. Their mode of action is somehow original in the panorama of engulfment receptors since they act as lipid transporters. While in the worm the loss of CED-7 has phenotypic consequences exclusively on engulfment, in the mouse the deletion of

Article available at http://www.biologie-journal.org or http://dx.doi.org/10.1051/jbio:2005020

ABCA1 by homologous recombination has highlighted broad consequences on macrophage biology.

Among those we will discuss here the aberrant responses of ABCA1$^{-/-}$ mice to *Plasmodium berghei* ANKA infection, concerning in particular the development of cerebral malaria (CM), a cytokine-induced immunopathology. This syndrome involves a central role of monocytes and, as shown recently, high levels of circulating microparticles. It was found that ABCA1 loss completely protects against CM and its associated mortality. This observation, together with the demonstration of quantitative and functional modifications of microparticles, suggests that microparticles may be involved in CM pathogenesis. The ABCA1 transporter thus appears to control susceptibility to CM, thereby providing new insights in its pathophysiological mechanisms and potential new therapeutic avenues.

Programmed cell death is a major control pathway of cell homeostasis both during development and in physiological and pathological conditions. It can be schematically split into two phases. The first phase generates the progressive dismantling of cells: its circuits and principal effectors being largely studied and well known (Zwaal & Schroit, 1997; Fadok *et al*., 1998; Hengartner, 2001; Schlegel & Williamson, 2001). This is followed by the clearance of dying corpses via a specialised form of phagocytosis, called engulfment (Alibert & Chimini, 2002; Reddien & Horvitz, 2004).

Engulfment is indeed peculiar in the world of phagocytosis (Chimini & Chavrier, 2000; Savill *et al*., 2002). First and most important the process is self centered the prey being a dying self cell and not a foreign body. Second it is constantly active since the generation of dead cells is a continuous process and, finally, it is immunological silent. The immune silence primarily results from the swiftness of the clearance that occurs well before leakage of dangerous contents from the dying cells. Moreover it triggers active anti-inflammatory signalling from the scavenging macrophage and this is in sharp contrast to *bona fide* phagocytosis, which signals danger by definition.

Finally, it is worth remembering that while engulfment is primarily the task of professional phagocytes, the macrophages, it may be acted, though less efficiently, by amateurs phagocytes such as dendritic cells, fibroblasts or hepatocytes (Dini *et al*., 1995; Albert *et al*., 1998; Wood *et al*., 2000).

FROM RECOGNITION TO INGESTION: A MEMBRANE EVENT WITH MULTIPLE FACETS

Several steps are required to achieve efficient engulfment. Recruitment of macrophages to the site of death is essential and is potentially due to positive chimiotactism. Little is however known of the factors implied; a single report highlighted a positive effect of lysophosphatidylcholine on macrophage recruitment (Lauber *et al*., 2003; Ravichandran, 2003). However considering

TABLE I. – Surface receptors involved in the recognition of apoptotic cells and their ligand specificity.

Receptor	Structural family	Organism	Ligand on apoptic cells	Other ligands	Reference
SR-A	Scavenger receptor class A	Mammals	?	OXLDL AcLDL OxHDL	(Platt *et al*., 1996)
SR-BI	Scavenger receptor class B	Mammals	Thrombospondin (with αvβ3) ?	Gram$^{+/-}$ bacteria HDL AcLDL OxLDL Anionic phospholipids VLDL	(Krieger, 2001)
CD36				OxLDL HDL Anionic phospholipids	(Febbraio *et al*., 2001) (Savill *et al*., 1992)
CED-1	Scavenger receptor class F	*C. elegans*	?	?	(Zhou *et al*., 2001)
CD14	GPI linked receptor	Mammals	ICAM3 ?	LPS of Gram-bacteria-	(Gregory, 2000)
PSR ?	PS	Mammals	Phosphatidylserine	?	(Fadok *et al*., 2000)
MER	Receptor tyrosine kinase	Mammals	Gas-6	?	(Scott *et al*., 2001)
αvβ3/αvβ5	Integrins	Mammals	Thrombospondin (with CD36)	Vitronectin	(Savill *et al*., 1992) (Albert *et al*., 1998)
?	Lectins	Mammals	Glycosylated	Carbohydrates	(Duvall *et al*., 1985) (Savill *et al*., 1989)

the late release of this lipid from dying cells in a context where swiftness is essential, lysophosphatidylcholine is unlikely to be the sole or the influential soluble factor at play.

Once on the site of death, the macrophages have to recognize suitable preys among healthy cells. How this happens is still uncertain. The existence of negative or positive tags on the doomed cells has been evocated, consistent with the idea that the engagement in the apoptotic pathway either disables signals for cell fitness or triggers the appearance of specific signs (Chimini, 2002). In both cases cell interaction is required but whereas in the negative hypothesis the absence of normal repulsive signalling permits prolonged interaction, in the second the contact is directly induced by specific receptor-ligand recognition. Homotypic CD31 signalling and its disabling during apoptosis is the unique example supporting negative sorting (Brown *et al*., 2002). On the contrary, for positive sorting, numerous are the modifications induced by death on cell membranes, as are numerous the candidate receptors on the macrophage surface (Fadok & Chimini, 2001; Alibert & Chimini, 2002). They have been extensively reviewed elsewhere and are reported schematically in Table I.

It is however important to remark the fact that most if not all the candidate engulfment proteins are multi-ligand receptors in that they are able to recognize with low affinity a number of ligands of various nature (Krieger & Stern, 2001). This suggests that engulfment, in contrast to other forms of phagocytosis, has not developed specific and affine recognition systems but, rather, derives at its benefits a number of pre-existing molecules. In other words, redundancy and multiplicity have been favoured for this type of cell interaction instead of high affinity and unicity. This, while being an effective safety measure, implies experimental difficulty for an appro-

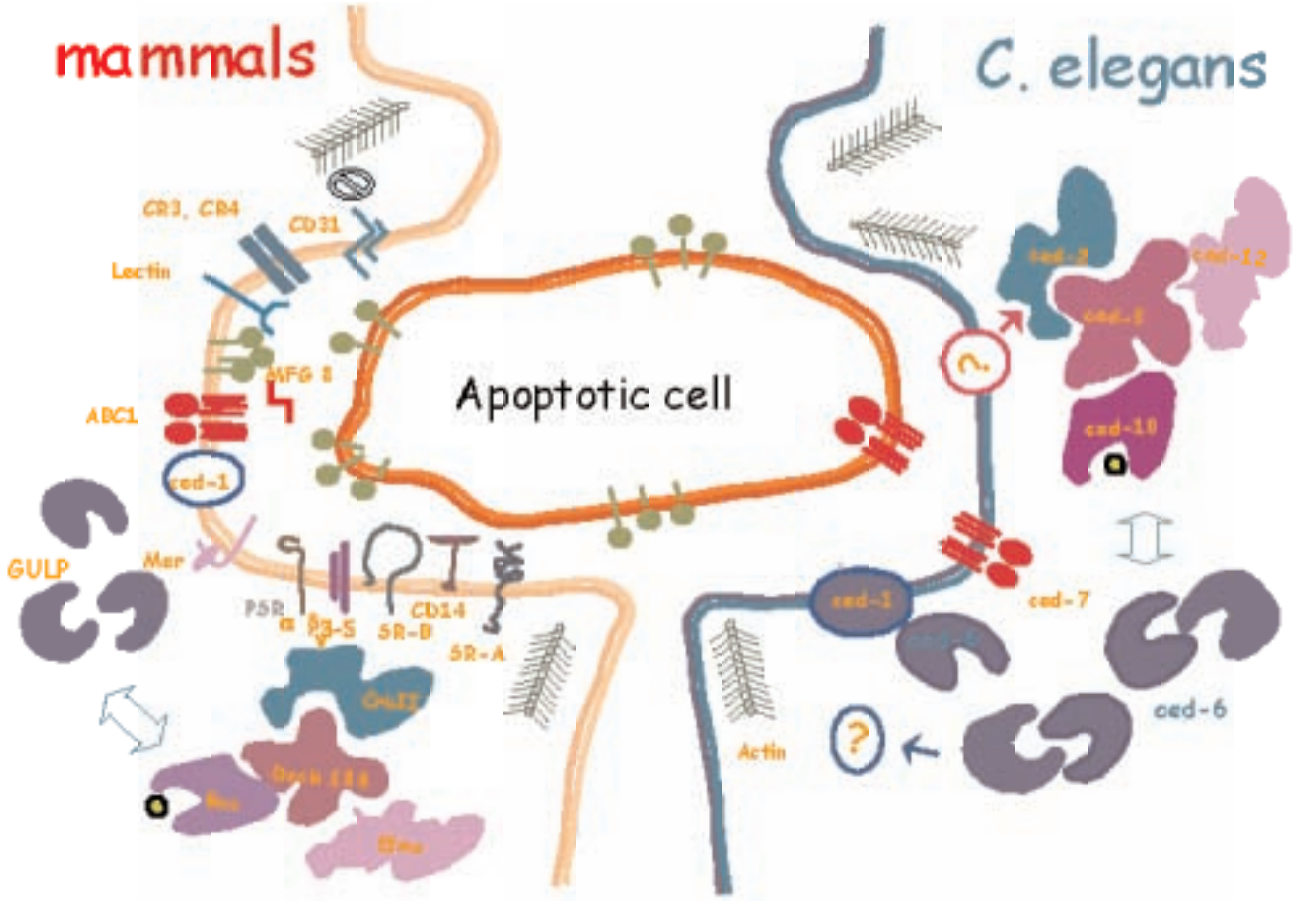

FIG. 1. – *Surface receptors and signalling molecules involved in engulfment.*

The two model systems (*C. elegans* and mammals) are schematized. Green dots on the surface of apoptotic cells represent exposed PS On the mammalian side a number of surface receptors haves been identified . Details on their interactions with the apoptotic cells are reported in Table I. Receptor engagement is expected to trigger downstream signalling cascades, leading to the final ingestion (Riedden & Horvitz, 2004). These have been detailed in *C. elegans* for the pathway defined by CED-2, CED-5, CED-12 and CED-10. No membrane receptor has been identified along this pathway in the nematode. It corresponds in mammals to the cascade triggered by integrins and leading to progressive recruitment in the same molecular complex CrKII, DOCK180, ELMO and Rac.

To the second pathway belong CED-1, CED-7 and CED-6. Their molecular interactions are not clear. While the mammalian ortholog of CED-1 is still uncertain, ABCA1 and GULP have been validated as orthologs of CED-7 and CED-6.

priate study of the detrimental consequences of impaired engulfment.

Indeed in most cases the generation of knock out animals carrying a deletion of single receptor revealed engulfment impairments but limited immunopathological consequences (Trigatti *et al*., 1999; Febbraio *et al*., 2000; Platt *et al*., 2000; Scott *et al*., 2001; Hanayama *et al*., 2004).

Efficient recognition is followed by ingestion, which is acted by downstream effectors and leads to actin assembly and phagosome formation. Again the literature is extensive on the subject and we will simply present here a schematic recapitulation of the process in Figure 1 (Fadok & Chimini, 2001; Reddien & Horvitz, 2004). For the sake of clarity we report and compare in the figure the two major models for the study of engulfment, the nematode *C. elegans* and the mammalian system.

ABCA1 IN THE FRAMEWORK OF ENGULFMENT

The ATP binding cassette (ABC) transporters of the A subclass, ABCA1 and its ortholog CED-7 in the nematode, are somehow unique cases among engulfment receptors. In fact in contrast to all the others they have been identified independently and simultaneously in the two model systems (Luciani & Chimini, 1996; Wu & Horvitz, 1998). On the basis of extensive analysis of the molecular function in in vitro reconstitution systems ABCA1 is considered to be a transporter of lipids, a property shared by all transporters of the A class (Dean *et al*., 2001; Hamon *et al*., 2002; Holland *et al*., 2003). A similar function can be surmised for CED7. Indeed ABCA1 functions at the plasma membrane as a flippase of phosphatydylserine (PS) with the net result of increasing the amount of this lipid in the outer leaflet (Hamon *et al*., 2000). PS are anionic phospholipids normally actively restricted to the inner leaflet of the membrane. Even a minimal outward flip of PS is thus highly destabilizing and rich of influences on the general physicochemical properties of the membrane (Sprong *et al*., 2001). It is known in fact that lateral and transversal distribution of individual phospholipid species is a major determinant both of the intrinsic bending ability of membranes and of lateral distribution or mobility of membrane proteins

In the context of engulfment this translates into a facilitator function for ABC-A transporters, related to their ability to destabilise membrane architecture. The latter would favour the local recruitment of receptors in a sort of synaptic cleft increasing the efficiency of signalling. This has been visualized in the nematode, where the absence of CED-7 hampers clustering of the receptor CED-1 in the phagocytic cup (Zhou *et al*., 2001).

The *bona fide* requirement for ABCA1 during engulfment has been evidenced *in vitro* and formally reinforced by the analysis of knock out mice, which recapitulate perfectly the CED-7 phenotype of the worm (Hamon *et al*., 2000). However the absence of ABCA1 has more profound consequences than a mere persistence of dead cells. In fact and consistent with the role of ABCA1 as a lipid transporter, the mice develop major dysfunctions of lipid metabolism known in humans as Tangier disease (Young & Fielding, 1999). This genetic disorder is characterized by an impaired reverse transport of cholesterol from peripheral cells to the liver for final catabolism (Assmann, 2001). Major consequence of this is an elevated risk for cardiovascular disorders due to increased cellular content of cholesterol and development of atheromatous lesions. On this basis and considering that macrophages are in both cases central to the phenotypic abnormality we set out to analyse unexpected immunological consequences in the ABCA1 knock out animal model.

THE LOSS OF ABCA1 TROUBLES THE LIFE OF A MACROPHAGE: A PARADIGMATIC EXAMPLE

We were interested to evaluate the consequence of the loss of ABCA1 in a mouse model of cerebral malaria (CM), for two reasons. First, this syndrome, characterised by a neurovascular pathology, clearly involves numerous features of monocyte /macrophage biology in its pathogenesis (Grau *et al*., 1988; Hunt & Grau, 2003), as summarised in Figure 2; second, it has become clear recently that microparticles (MP) are dramatically elevated in patients with CM (Combes *et al*., 2004). The functional link between monocyte activation and MP production lies in the cytokine TNF, the overproduction of which has been documented, both in experimental and human CM (Grau *et al*., 1987, 1989). As a matter of fact, activation of endothelial cells by TNF causes a significant enhancement of membrane vesiculation, resulting in the release of increased numbers of MP (Combes *et al*., 1999).

As a first approach to an experimental appraisal of ABCA1 in CM, we quantitated circulating levels of MP at the time of the neurological syndrome. Exactly paralleling the observations in Malawian patients (Combes *et al*., 2004), mice that are known to be genetically susceptible to the neurological syndrome presented a significant rise in circulating MP levels, on day 7 after infection with *Plasmodium berghei* ANKA (PbA), compared to pre-infection levels (Fig. 3A). This was seen both in outbred (Swiss) and inbred (C57BL/6) mice. As shown in Figure 3B, platelets represented the major cell source among circulating MP, while monocytes and endothelial cells accounted each for less than 25 % of these MP. Malarial infection did not modify the proportion of platelet, monocytic and endothelial MP in circulation. An exacerbation of membrane vesiculation thus represents yet another parameter in common between murine and human CM, in addition to those which have been described, on the histopathological, neurochemical and immunological points of view (for review, see (Lou *et al*., 2001; de Souza & Riley, 2002; Hunt & Grau, 2003; Coltel *et al*., 2004).

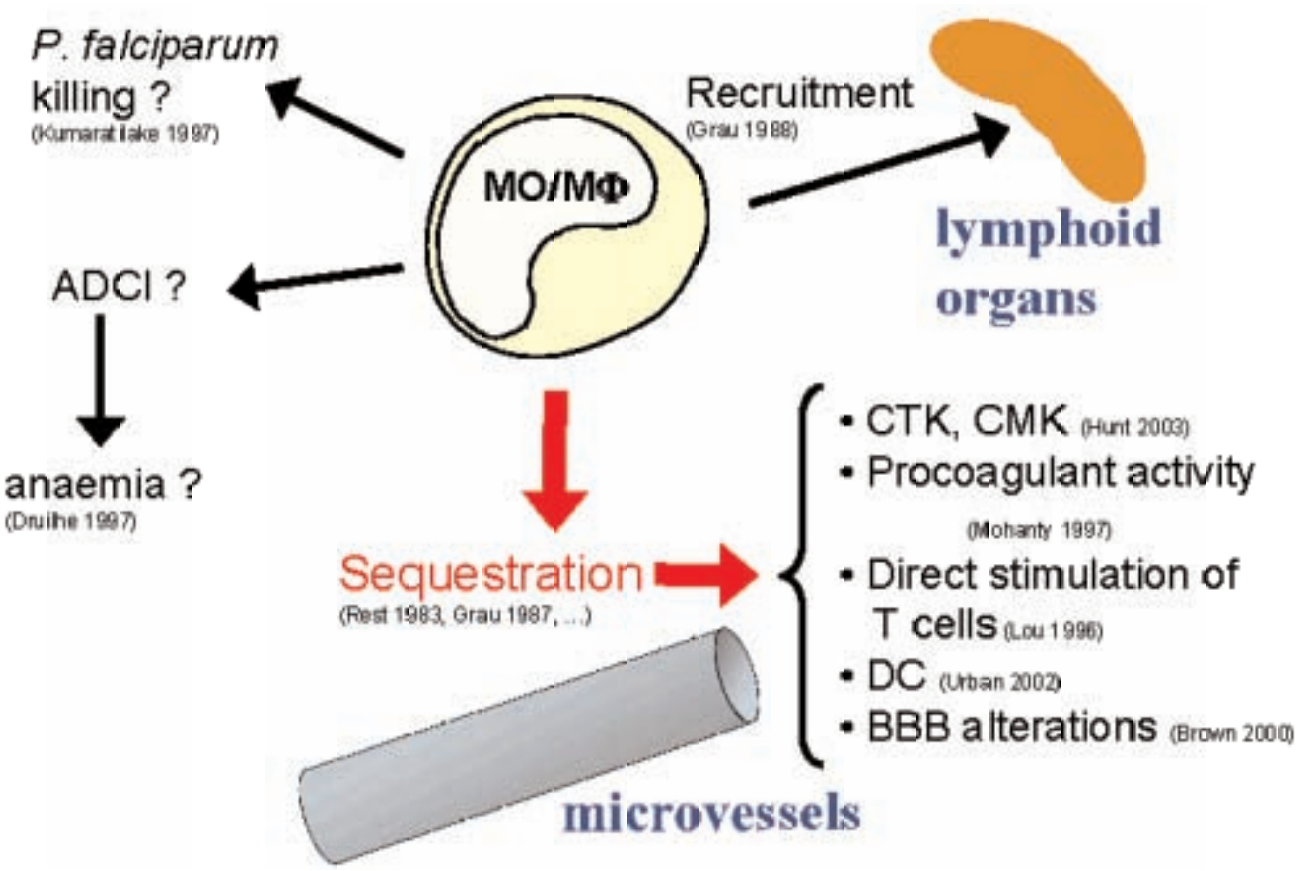

FIG. 2. – *Some aspects of monocyte involvement in malarial pathogenesis*.

Monocytes/macrophages (MO/MF) may have direct and indirect effects that are instrumental in malarial complications. Monocytes play a role in the killing of the parasite and via antibody-dependent inhibition (ADCI), involving the crosslinking of monocytes and merozoites by cytophilic antibodies, may be partly responsible for the development of chronic anaemia. In experimental CM, a massive recruitment of monocytes in lympoid organs, essentially the spleen, represented diagrammatically here, and lymph nodes, is brought about by IL-3 and GM-CSF. These monocytes in increased numbers participate in systemic overproduction of cytokines, notably TNF (Grau *et al*., 1988). Numerous pathophysiological consequences arise from locally arrested monocytes in microvessels, notably in brain and lung, summarised here (Rest, 1983; Lou *et al*., 1996; Druilhe & Perignon, 1997; Kumaratilake *et al*., 1997; Mohanty *et al*., 1997; Brown *et al*., 2000; Urban & Roberts, 2002). CTK: cytokines; CMK: chemokines; DC: dendritic cells; BBB: blood-brain barrier; MO/MΦ: monocytes macrophages.

In view of this, we then used this mouse model to estimate the possibility that the loss of ABCA1 gene could alter the expression of cerebral pathology in malaria-infected animals. As can be seen on Figure 3C, ABCA1 KO mice were completely protected against CM and its associated mortality, while heterozygous ABCA1$^{+/-}$ mice presented the same incidence of neurological syndromes than their wild-type counterparts, on a DBA/1 background (Combes *et al*., 2005).

Thus, the sole absence of the ABCA1 gene converts the susceptibility of the DBA/1 strain of mice to complete resistance to CM. However, this complete protection might have been due to an effect of the gene deletion on the course of infection and/or to the massive hypolipaemia which also results from ABCA1 inactivation. The first possibility was ruled out by the demonstration of strictly identical curves of parasitaemia in PbA-infected ABCA1 $^{-/-}$ and $^{+/+}$ mice. The second possible confounding factor also was unlikely, since Apo-A1 KO mice, which are equally hypolipaemic, were not protected at all against CM. Thus, the intrinsic activity of ABCA1 gene, rather than its metabolic consequences, is to be considered causal in the reversion of susceptibility of DBA/1 mice to CM.

The cellular and molecular mechanisms by which ABCA1 deletion confers this complete protection against CM were then investigated. We first focused our attention on the hallmarks of CM in terms of histopathology: the sequestration of mononuclear leucocytes and the activation of brain microvascular endothelial cells. On brain specimens sampled on day 7 of infection, the expression of both parameters was significantly lower in PbA-infected ABCA1 KO mice than in infected wild-type animals. The brain accumulation of intravascular monocytes was eradicated in infected ABCA1 KO mice, *i.e*., these cells were not more numerous than in the brain of non-infected mice. Similarly, on brain microvessels from infected ABCA1 mice, quantitative image analysis indicated that

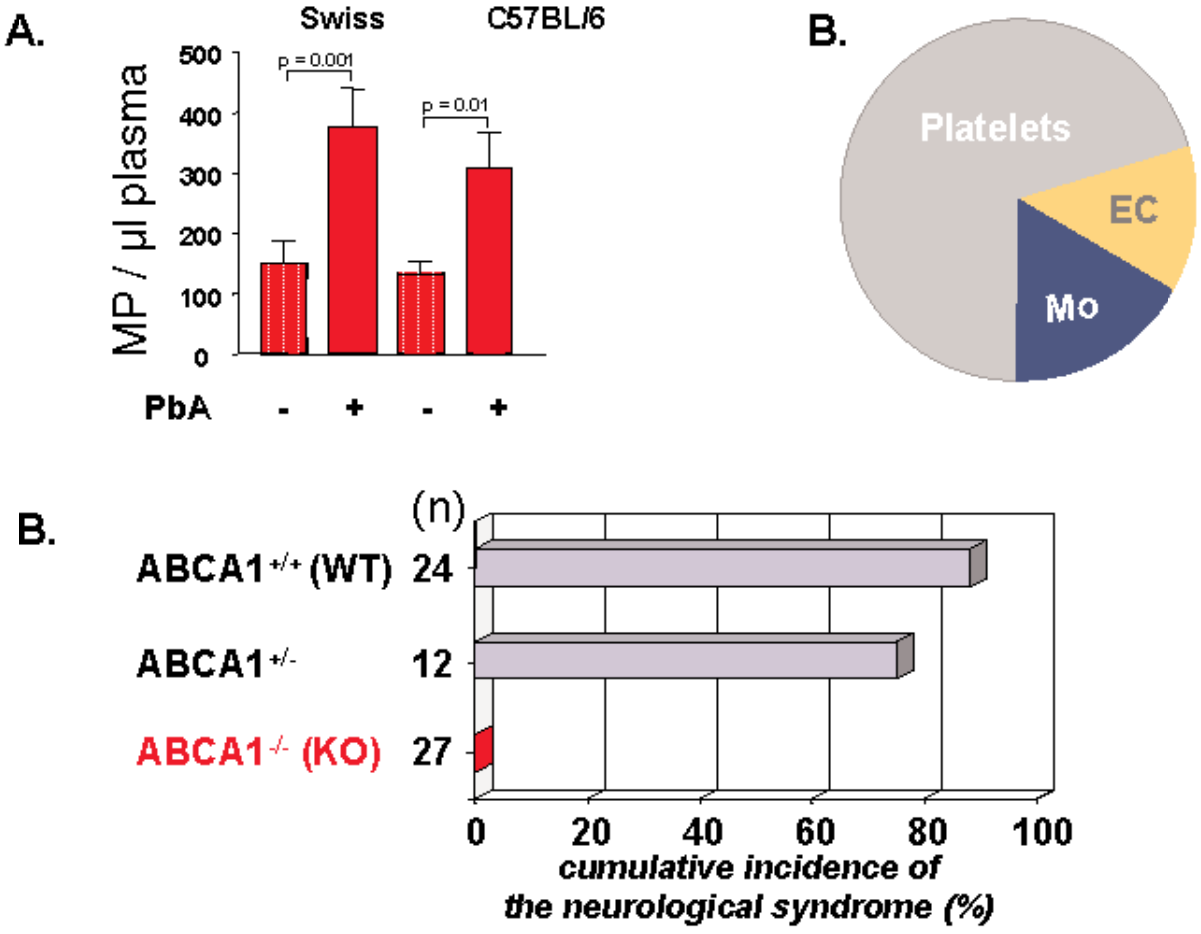

FIG. 3 – *MP levels are higher at the time of CM in genetically susceptible,PbA-infected mice, and ABCA1 gene deletion fully prevents cerebral lesions.*

A. Circulating levels on day 7 of infection. B. Cellular origin of circulating MP, as assessed by flow cytometry, as described (Combes *et al*., 2005). C. Cumulative incidence of CM-associated mortality in wild-type ($^{+/+}$); heterozygous ($^{+/-}$) and homozygous ($^{-/-}$) ABCA1 KO mice. n = number of animals.

there was a markedly reduced upregulation of ICAM-1 and VCAM-1, two molecules which consistently reflect microvascular alterations in CM (Grau *et al*., 1991). The intracerebral expression of TNF also was significantly lower in ABCA1 KO than in wild type mice, essentially at the level of vascular structures, since the parenchymal expression of the cytokine, notably in the microglial compartment, did not appear to be modified. Finally, quantitative immunohistopathology demonstrated that the accumulation of intravascular platelets, which invariably accompanies CM, both in the mouse model and in patients who died of the syndrome (Stoelcker *et al*., 2002; Grau *et al*., 2003), was significantly less marked in malaria-infected ABCA1 mice than in wild-type DBA/1.

The extent to which a down-modulation of MP production was associated to resistance to CM was addressed. It was clear that the during malaria infection, ABCA1 KO mice indeed presented significantly lower MP levels than wild-type animals ($p = 0.027$). Similarly, when cells were isolated from ABCA1 KO mice and studied in in vitro re-stimulation assays, the loss of ABCA1 gene was accompanied by a lower reactivity of cells in response to classical agonists of cellular vesiculation, as assessed by lower numbers of MP released in culture supernatants. The MP production capacity was significantly reduced in ABCA1 macrophages, and abolished in ABCA1 platelets. Besides drastic modifications in numbers of MP released in the plasma during malaria and in cultures, the functional properties of the MP produced also appeared to differ. Using classical cytokine-release assays from macrophages and chronographic assays of clotting time, it was found that the malarial-induced MP in ABCA1 mice are less pro-inflammatory and less procoagulant that those produced in CM-susceptible wild-type animals.

This impaired MP production may thus be of pathogenic significance in CM. As a non mutually exclusive alternate explanation, the ABCA1 gene deletion may be protecting against CM *via* the reduced TNF production

capacity of monocytes/macrophages or via a yet to be defined parameter in the biology of these phagocytes. Several lines of experiments are still required to delineate the respective importance of these pathways.

In conclusion unforeseen phenotypic alterations of macrophages have been detected in the ABCA1$^{-/-}$ animals. Those broader than expected functional outcomes, which largely prevail over impaired engulfment, must be interrelated. One possibility is that they can all be accounted for by the loss of the dynamic properties of macrophage membrane, *per se* able to interfere mechanistically with a broad range of signalling pathways. Alternatively the animal may have exacerbated feed back circuits to balance life threatening outcomes. At any rate nature has once again efficiently buffered the major threat of impaired engulfment, *i.e.*, the loss of immune surveillance and the development of autoimmune disease.

REFERENCES

Albert M. L., Pearce S. F. *et al*., Immature dendritic cells phagocytose apoptotic cells *via* alphavbeta5 and CD36, and crosspresent antigens to cytotoxic T lymphocytes. *J. Exp. Med*., 1998, *188*, 1359-1368.

Alibert M. & Chimini G., L'élimination des cellules apoptotiques : une phagocytose particuliére. *Médecine/sciences*, 2002, *18*, 853-860.

Assmann G., von Eckardstein A., Brewer H. B., Familial analphalipoproteinemia: Tangier disease. The Metabolic Basis of Inherited Disease. Scriver C. R., Beaudet A. L., Sly W. S., Valle D. New York, McGraw-Hill, 2001, 2937-2960.

Brown H. C., Chau T. T. *et al*., Blood-brain barrier function in cerebral malaria and CNS infections in Vietnam. *Neurology*, 2000, *55*, 104-111.

Brown S., Heinisch I. *et al*., Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. *Nature*, 2002, *418*, 200-203.

Chimini G., Apoptosis: repulsive encounters. *Nature*, 2002, *418*, 139-141.

Chimini G. & Chavrier P., Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. *Nat. Cell Biol*., 2000, *2*, E191-E196.

Coltel N., Combes V. *et al*., Cerebral malaria – a neurovascular pathology with many riddles still to be solved. *Curr. Neurovasc. Res*., 2004, *1*, 91-110.

Combes V., Coltel N. *et al*., ABCA1 gene deletion protects against cerebral malaria: potential pathogenic role of microparticles in neuropathology. *Am. J. Pathol*., 2005, *166*, 295-302.

Combes V., Simon A. C. *et al*., *In vitro* generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. *J. Clin. Invest*., 1999, *104*, 93-102.

Combes V., Taylor T. E. *et al*., Circulating endothelial microparticles in malawian children with severe falciparum malaria complicated with coma. *JAMA*, 2004, *291*, 2542-2544.

de Souza J. B. & Riley E. M., Cerebral malaria: the contribution of studies in animal models to our understanding of immunopathogenesis. *Microbes Infect.*, 2002, *4*, 291-300.

Dean M., Hamon Y. *et al*., The human ATP-binding cassette (ABC) transporter superfamily. *J. Lipid Res*., 2001, *42*, 1007-1017.

Dini L., Lentini A., *et al*., Phagocytosis of apoptotic bodies by liver endothelial cells. *J. Cell Sci*., 1995, *108*, 967-973.

Druilhe P. & Perignon J. L., A hypothesis about the chronicity of malaria infection. *Parasitol Today*, 1997, *13*, 353-357.

Duvall E., Wyllie A. H. *et al*., Macrophage recognition of cells undergoing programmed cell death (apoptosis). *Immunology*, 1985, *56*, 351-358.

Fadok V., Bratton D. L. *et al*., The role of Phosphatidylserine in recognition of apoptotic cells by phagocytes. *Cell Death Differ*., 1998, *5*, 551-562.

Fadok V. A., Bratton D. L. *et al*., A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature*, 2000, *405*, 85-90.

Fadok V. A. & Chimini G., The phagocytosis of apoptotic cells. *Semin. Immunol*., 2001, *13*, 365-372.

Febbraio M., Hajjar D. P. *et al*., CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J. Clin. Invest*., 2001, *108*, 785-791.

Febbraio M., Podrez E. A. *et al*., Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *J. Clin. Invest*., 2000, *105*, 1049-1056.

Grau G. E., Fajardo L. F. *et al*., Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. *Science*, 1987, *237*, 1210-1212.

Grau G. E., Kindler V. *et al*., Prevention of experimental cerebral malaria by anticytokine antibodies. Interleukin 3 and granulocyte macrophage colony-stimulating factor are intermediates in increased tumor necrosis factor production and macrophage accumulation. *J. Exp. Med*., 1988, *168*, 1499-1504.

Grau G. E., Mackenzie C. D. *et al*., Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. *J. Infect. Dis.*, 2003, *187*, 461-466.

Grau G. E., Pointaire P. *et al*., Late administration of monoclonal antibody to leukocyte function-antigen 1 abrogates incipient murine cerebral malaria. *Eur. J. Immunol.*, 1991, *21*, 2265-2267.

Grau G. E., Taylor T. E. *et al*., Tumor necrosis factor and disease severity in children with *falciparum malaria*. *N. Engl. J. Med.*, 1989, *320*, 1586-1591.

Gregory C. D., CD14-dependent clearance of apoptotic cells: relevance to the immune system. *Curr. Opin. Immunol.*, 2000, *12*, 27-34.

Hamon Y., Broccardo C. *et al*., ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. *Nat. Cell Biol*., 2000, *2*, 399-406.

Hamon Y., Chambenoit O. *et al*., ABCA1 and the engulfment of apoptotic cells. *Biochim. Biophys. Acta*, 2002, *1585*, 64-71.

Hanayama R., Tanaka M. *et al*., Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science*, 2004, *304*, 1147-1150.

Hengartner M. O., Apoptosis: corralling the corpses. *Cell*, 2001, *104*, 325-328.

Holland I. B., Cole S. P. C. *et al*., Eds. ABC proteins. from bacteria to man. London, san Diego, Academic Press, 2003.

Hunt N. H. & Grau G. E., Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol.*, 2003, *24*, 491-499.

Krieger M., Scavenger receptor class B type I is a multiligand HDL receptor that influences diverse physiologic systems. *J. Clin. Invest*., 2001, *108*, 793-797.

Krieger M. & Stern D. M., Series introduction: multiligand receptors and human disease. *J. Clin. Invest*., 2001, *108*, 645-647.

Kumaratilake L. M., Ferrante A. *et al*., The role of complement, antibody, and tumor necrosis factor alpha in the killing of *Plasmodium falciparum* by the monocytic cell line THP-1. *Infect. Immun.*, 1997, *65*, 5342-5345.

Lauber K., Bohn E. *et al*., Apoptotic cells induce migration of phagocytes *via* caspase-3-mediated release of a lipid attraction signal. *Cell*, 2003, *113*, 717-730.

Lou J., Dayer J. M. *et al*., Direct cell/cell contact with stimulated T lymphocytes induces the expression of cell adhesion molecules and cytokines by human brain microvascular endothelial cells. *Eur. J; Immunol*., 1996, *26*, 3107-3113.

Lou J., Lucas R. *et al*., Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. *Clin. Microbiol. Rev.*, 2001, *14*, 810-820, table of contents.

Luciani M. F. & Chimini G., The ATP binding cassette transporter ABC1, is required for the engulfment of corpses generated by apoptoic cell death. *EMBO J*., 1996, *15*, 226-235.

Mohanty D., Ghosh K. *et al*., Fibrinolysis, inhibitors of blood coagulation, and monocyte derived coagulant activity in acute malaria. *Am. J. Hematol*., 1997, *54*, 23-29.

Platt N., Suzuki H. *et al*., Apoptotic thymocyte clearance in scavenger receptor class A-deficient mice is apparently normal. *J. Immunol*., 2000, *164*, 4861-4867.

Platt N., Suzuki H. *et al*., Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes *in vitro*. *Proc. Natl. Acad. Sci. USA*, 1996, *93*, 12456-12460.

Ravichandran K. S., "Recruitment signals" from apoptotic cells: invitation to a quiet meal. *Cell*, 2003, *113*, 817-820.

Reddien P. W. & Horvitz H. R., The engulfment process of programmed cell death in *Caenorhabditis elegans*. *Annu. Rev. Cell Dev. Biol.*, 2004, *20*, 193-221.

Rest J. R., Pathogenesis of cerebral malaria in golden hamsters and inbred mice. *Contrib. Microbiol. Immunol.*, 1983, *7*, 139-146.

Savill J., Dransfield I. *et al*., A blast from the past: clearance of apoptotic cells regulates immune responses. *Nat. Rev. Immunol.*, 2002, *2*, 965-975.

Savill J., Hogg N. *et al*., Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J. Clin. Invest*., 1992, *90*, 1513-1522.

Savill J. S., Henson P. M. *et al*., Phagocytosis of aged human neutrophils by macrophages is mediated by a novel "charge-sensitive" recognition mechanism. *J. Clin. Invest*., 1989, *84*, 1518-1527.

Schlegel R. A. & Williamson P., Phosphatidylserine, a death knell. *Cell Death Differ.*, 2001, *8*, 551-563.

Scott R. S., McMahon E. J. *et al*., Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature*, 2001, *411*, 207-211.

Sprong H., van der Sluijs P. *et al*., How proteins move lipids and lipids move proteins. *Nat. Rev. Mol. Cell Biol*., 2001, *2*, 504-513.

Stoelcker B., Hehlgans T. *et al*., Requirement for tumor necrosis factor receptor 2 expression on vascular cells to induce experimental cerebral malaria. *Infect. Immun*., 2002, *70*, 5857-5859.

Trigatti B., Rayburn H. *et al*., Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc. Natl. Acad. Sci. USA*, 1999, *96*, 9322-9327.

Urban B. C. & Roberts D. J., Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells. *Curr. Opin. Immunol*., 2002, *14*, 458-465.

Wood W., Turmaine M. *et al*., Mesenchymal cells engulf and clear apoptotic footplate cells in macrophageless PU.1 null mouse embryos. *Development*, 2000, *127*, 5245-5252.

Wu Y. & Horvitz R. H., The *C. elegans* cell corpse engulfment gene ced-7 encodes a protein similar to ABC transporters. *Cell*, 1998, *93*, 951-960.

Young S. G. & Fielding C. J., The ABCs of cholesterol efflux. *Nature Genet*., 1999 *22*, 316-318.

Zhou Z., Hartwieg E. *et al*., CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans*. *Cell*, 2001, *104*, 43-56.

Zwaal R. F. A. & Schroit A. J., Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood*, 1997, *89*, 1121-1132.