Research Article

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Profile of Natural Killer (NK) Cells in Simple Malaria in Adults

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ABSTRACT

Justification: *In the problematic of protection against severe forms of malaria, premunition has often been mentioned as a protective factor acquired in adults at the cost of multiple infections for several years. Exploration of the cellular component of anti-parasite immunity in uncomplicated malaria will provide comparisons of evidence that, despite relative protection, 2 to 3% of adults living in the endemic zone are victims of severe malaria.*

Main objective: *The objective was to evaluate the role of the innate cellular response in susceptibility to uncomplicated malaria in subjects older than 15 years.*

Patients and Methods: *It was a prospective study with descriptive and analytical purpose that took place at Koumassi General Hospital for simple malaria patients and the NBTC for witnesses. All blood samples were analyzed in the Immunology and Hematology Laboratory of CHU de Cocody. It included 50 patients (25 patients with malaria and 25 witnesses) of both sexes, over a 3-month period. The samples carried were processed in the said-laboratory.*

Results: *The average age of our patients was 35 years. The mean of NK cells were 45 cells/mm^3 in patients and 154.64 cells/ mm^3 in witness persons.*

The risk of not seeing a simple malaria when the number of NK cells is high was 9.03. The PPV was 88.88% and the NPV was 62.6%. The mean parasitemia in patients was 1840 trophozoites/µL.

The influence of NK cells on parasitemia was undetermined with a PPV at 1% and a NPV at 39.13%.

Conclusion: *Susceptibility to simple malaria is a multifactorial phenomenon in which the immune response plays a central role. The evolution towards this clinical state will have to be studied with all the other cellular actors to better appreciate the role of NK cells during its evolution.*

Keywords

Malaria, NK cells, Innate immunity, Côte d'Ivoire.

Abbreviations

NBTC: National Blood Transfusion Center; NK: Natural Killer; NPV: Negative Predictive Value PPV: Positive Predictive Value, CHU: University Hospital Center; PBS: Phosphate Buffered Saline.

Introduction

Malaria, a disease caused by Plasmodium haematozoa, remains a major public health problem in all tropical and subtropical regions. There are five species, but only *Plasmodium falciparum* is redoubtable and deadly [1]. According to the latest WHO estimates, there were 212 million new cases of malaria worldwide and 429,000 deaths in 2015. Sub-Saharan Africa bears a disproportionate share

of this global burden of malaria with 88% of cases and 92% deaths [2]. In Côte d'Ivoire, malaria is hyper-endemic and is transmitted throughout the year with a recrudescence during the rainy seasons. The clinical course of the infection depends on several factors [3,4] including the immune status of the infected subject. Thus, the humoral immunity which is partly directed against the antigens of the parasite constitutes a so-called premunition step and remains partially protective [5]. It is gradually established for several years, following multiple infections [6,7]. In the wake of this partial protective immunity, a study conducted in Abidjan [8] mentioned the importance of NK cytotoxic cells in the pathophysiology of simple forms that are still the subject of many studies for its full understanding. Concerning the cytotoxic cellular profile, the question that often arises is the interest of NK cytotoxicity in the susceptibility to simple forms of malaria in endemic areas. [8] As part of an analytical approach to hospital malaria, we focused on NK, an immunocompetent cell. This cytotoxic cell, which is involved in the natural defense mechanisms directed against several pathogens, could intervene in the pathogenesis of human malaria even if this is still controversial. Indeed, experiments using human spleen cells suggest that red blood cells parasitized by *Plasmodium falciparum* are negligible targets of CTL (Cytotoxic T Lymphocyte) and NK cells [9]. Hence the present study which has had the general objective of evaluating the role of the innate cellular response in the susceptibility to simple malaria in subjects over 15 years of age (adults).

Materials and Methods

Type and places of study

It was a prospective study with descriptive and analytical purpose, conducted over a period of 3 months (February to April 2016). Patients were recruited at Koumassi General Hospital for cases and at the Treichville National Blood Transfusion Center (CNTS) for witnesses after obtaining authorization from the Ethics Committee. The purpose of the study was to explain to the patients the use to participate in that study. Those who agreed to participate were giving a fact sheet with information to be filled in. Writing informed consent was obtained in all cases from study participants and patients were selected according to the definition criteria for simple malaria.

Study population

Our study included subjects of all sexes, admitted to the targeted sites, over the study period. These included 50 subjects, including 25 cases of uncomplicated malaria (WHO criteria) and 25 witness persons defined as having been living in an endemic area consistently for at least 5 years, showing no clinical signs of malaria and having thick, negative drops.

Blood collection

Blood was collected by puncture of a peripheral vein after cleaning the surface with alcohol buffer, on three tubes: a tube without anticoagulant for the dosage of CRP, glycemia and a tube containing an anticoagulant, the EDTA to carry out NFS to look for platelets, hemoglobin and leukocyte levels to eliminate a bacterial infection. Finally, a last collection on another EDTA tube which allowed us to count NK cells. Blood smear and thick drop were carried out for respectively species confirmation and parasite density. All the samples were analyzed at the Laboratory of Immunology and Hematology of the University Hospital of Cocody.

Flow Cytometer Analysis

To obtain the different proportion of NK cell rates, we first carried out their isolation on Ficoll-isopaque, then immunostaining technique with specific antibodies coupled to fluorochromes (CD3 FITC / CD16 + 56 PE / CD45PerCP), BD reagent (Becton Dikinson). 3 ml of whole blood diluted in half in PBS buffer after reconstruction was based on a tube containing 3 ml of ficoll. After centrifugation at 4°C and 3000 tours per minute for 5 minutes, four layers were formed : the red cell layer, the layer containing ficoll solution, the mononuclear cell layer and the serum layer. Mononuclear cell layer was recollected, washed 3 times, then the pellet was taken up in one millimeter of PBS. The reading was then done with flow cytometry model: Facs Calibur serie E34297300578 version 002 manufacturer BD company and the analysis of the data acquired was carried out by Cell Quest Pro software.

Statistical analysis

Data was entered using Microsoft Excel software statistical analysis of those data was carried out using Epi Info software 3.5.2 version (2002). The comparison of percentages was carried out with Chi-2 Pearson test. The result was considered significant at the 5% level. The test of T Student was needed to compare the averages. The calculation of the Odds Ratio (O.R) enabled us to evaluate the influence of the NK cell rate in the occurrence of simple malaria.

Results

The mean age of our study population was 35.44 years with an average age of 38.36 years for our patients and 32.52 years for witnesses. The age group from 15 to 40 years predominated in both patients and witnesses (Figure 1). The parasitemia of our patients ranged from 1100 to 3100 trz / µL with an average of 1840 trz / µL (Figure 2). 64% had a lower than average parasitemia compared to only 36% who had higher parasitémie (Figure 3). The average of NK in witness patients was 154.64 elements/mm^3 against 45 elements/mm^3 in patients with a very significant difference (p = 0, 0000172) (Tableau I). In the under 40 years, this average was 148.10 elements / mm^3 for witnesses against 50.50 elements / mm^3 in patients. In the over 40s, it was 180.80 elements/mm^3 for witnesses and 38 elements/mm^3 for patients. The average of NK cells of the witness in the 2 age groups was higher than that of the patients. However, there was only a significant difference in the over 40s (Tableau II). The evaluation of the impact of the number of these NK cells on the clinical profile in Table III showed that, the risk of not seeing a simple malaria when the number of NK cells is high was 9.03 (Odds ratio). And that, in over 84% of cases, malaria did not occur when the rate of NK cells was high (PPV = 84, 61%). On the other hand, it occurred in more than 62% of cases if the rate of NK cells was low (VPN = 62.6%). As for the study of the impact of the number of NK cells on parasitemia in Table IV, it has been globally indeterminate (OR). It was the same among the

under 40s. On the other hand in the over 40 years, it was zero (OR = 0) with a VPP of 1% and a VPN of 50%.

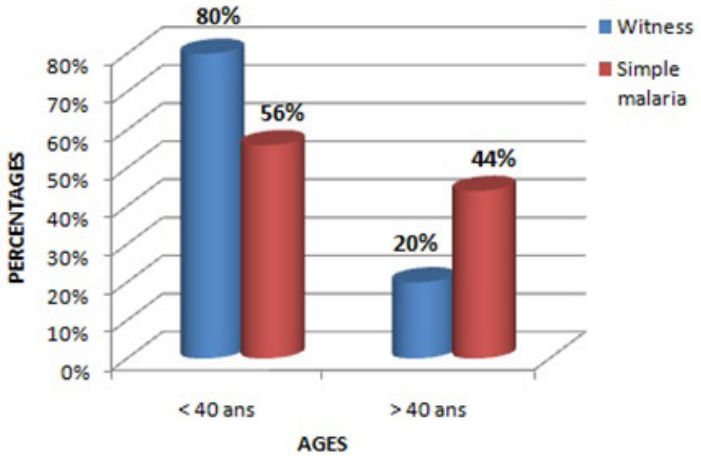

Figure 1: Distribution of the study population by age group.

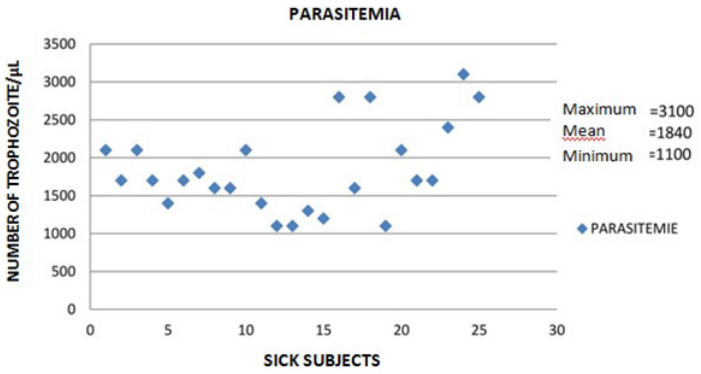

Figure 2: Distribution of parasitemia in sick subjects.

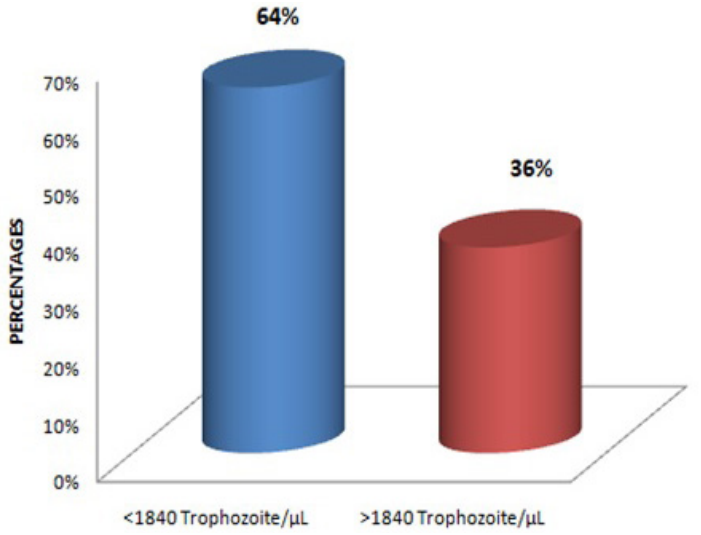

Figure 3: Distribution of cases of simple malaria according to the parasitemia.

Witness Subjects	Sick Subjects
154,64 elements/mm^3	45 elements/mm^3

Table 1: Comparison of NK cells means in witness subjects and in sick subjects. Chi 2 : p=0 ,0000172.

Age Groups	Witness Subjects	Sick Subjects	P
[15-40] years	148,1	50,5	p=0,08
[40-73] years	180,8	38	p=0,03

Table 2: Comparison of averages of NK cells in different age classes in witness subjects and in sick subjects.

NK	Witness Subjects	Sick Subjects	Total
NK > 154 cells	11	2	13
NK ≤ 154 cells	14	23	37
Total	25	25	50

Table 3: Evaluation of the impact of the number of NK cells on the clinical profile. Odds ratio= 9,03 PPV = 84 ,61% NPV = 62.6%.

NK	Parasitemia ≤ 1840 Trz/µL	Parasitemia > 1840 Trz/µL	Total
NK > 154 cells/µL	**2**	**0**	**2**
NK ≤ 154 cells/µL	**14**	**9**	**23**
TOTAL	**16**	**9**	**25**

Table 4: Evaluation of the impact of NK cells on parasitemia. Odds ratio= undetermined PPV = 1% NPV = 39,13%.

Discussion

Among the 50 subjects recruited, the results revealed that those under 40 years of age represented 56% of the population in the sick and 80% in the witness subjects and 44% and 20%, respectively, in the over 40s. The average age was 38.36 years for our sick population. Our results were roughly in the same proportions of 30 to 40 years as those of several authors [10-12]. For the recorded cases of simple malaria, parasitemia ranged from 1100 to 3100 trophozoites / µL of blood (with a thick drop). For a less parasitemia with *Plasmodium falciparum*, however, adult subjects showed signs of simple malaria. 64% of adults had parasitemia less than1840 Trz / µL. In our study, we did not find any significant difference in the distribution of parasitemia in different age groups. Indeed, work has shown that the development of parasites in certain types of immune cells can be prevented by the innate immune system. The innate mechanisms of inhibition of parasite growth by the human host would probably be the cause of the low rate of parasitemia observed in these acute *Plasmodium Falciparum* infections [13].

The study of the evolution of NK cells according to clinical status showed that the number of NK cells varied as much in the subjects with simple malaria as in the witness subjects. Comparatively, this rate was lower in uncomplicated malaria than in witness. There is little data in the literature related to a decline in NK cells activity in uncomplicated malaria. However, Ribero-Dias and coll [14] denounced an alteration of the cytotoxic function of NK cells during *Plasmodium falciparum* malaria. In another context of a murine model, Dogruman and coll [15] reported lower expression of NK markers in *P. berghei* infected mice than in healthy witnesses. Our results could be explained by the presence of premunition in adult subjects in malaria endemic areas [5]. This is based on cooperation between the parasite and circulating human antibodies at different stages, leading to antibody-dependent cellular inhibition (ADCI)

of intra-erythrocyte growth of the parasite. In contrast to the results of our study, NK cells appear to play an important role in the evolution of malaria [16]. Studies reveal that NK cells are among the first cells to respond to *Plasmodium falciparum* by producing IFN-gamma demonstrated by in vitro and in vivo studies [17]. These cells increase particularly in number [18]. This role of NK has also been emphasized by several authors during simple [17] and severe manifestations of malaria [16-19]. However, studies of the dynamics of NK cells activity in patients with malaria have revealed heterogeneous results, particularly in individuals infected with *P. falciparum* [17].

These contradictory results could be due to differences in the basic values of the absolute counts of the immune cells of the subjects of study. Otherwise, an impact of different geographic locations suggested by some authors might reflect such diverse results [15].

As for the study of the impact of NK cells on the occurrence of uncomplicated malaria, it emerges in a global way that the adult subject, whatever his age, is 9 times more likely not to make simple malaria when the rate of his NK cells is high. In addition, it appears that in 84.61% of cases, malaria does not occur when there is a high rate of NK cells. This figure is high compared to the negative predictive value (62%) meaning that in 62% of cases, malaria occurs when the level of NK cells is low. These overall figures are relatively similar to those found in the 15 to 40 age group. Better, subjects over the age of 40 were 15 times more likely to not see simple malaria occur when the number of NK cells was high. Furthermore, in 75% of cases, malaria did not occur when there was a high level of NK cells. This figure is low compared to the negative predictive value (83.33%) meaning that in 83.33% of cases, malaria occurs when the level of NK is low. Our results therefore indicate that NK cells are a risk, prediction and protective factor for uncomplicated malaria. This would probably be related to the fact that in the malaria-endemic area, malaria lowers the immune response of populations to malaria antigens [20].

Regarding the impact of NK cells on the evolution of parasitemia, the risk of having a low parasitemia when the level of NK cells is high was undetermined. We find the same results in subjects less than 41 years. This could be explained by the small size of our sample and the possible existence of a bias in the recruitment of our patients. But in subjects over 40, the risk of having low parasitemia when the NK cells level was high was nil with a low predictive value of NK cells (1%) on the evolution of parasitemia. These different results would be related to the control of parsitemia which is not only due to NK cells but also to the involvement of other cells of immunity. Indeed, in the cytotoxic effector response, generally and particularly in the anti-parasite immune response, the role of macrophages, cytotoxic T cells (TCD8 +) is also preponderant for the control of parasite multiplication.

Conclusion

Susceptibility to severe malaria is a multifactorial phenomenon in which the immune response plays a central role. The evolution towards this clinical state happens in certain situations through the simple form more or less symptomatic. Our results showed that NK cells are not specifically involved in this passage. On the other hand, in coordination with the whole immune system, they could play a role in anti-malarial immunity with regard to their low rate in patients compared to witnesses. It would therefore be important to study them with all the other cellular actors in order to better appreciate its role during the evolution of malaria infection.

References

1. Saxena QB, Biswas S, Sharma. Vp Interaction of human natural Killer cells with plasmodium falciparum erythrocytes. Exp Parasitol. 1989; 69-300.
2. www.who.int/features/factfiles/malaria/Fr/
3. Mazier D, Nitcheu J, Idrissa-Boubreu M. Cerebral malaria and immunogenetics Parasite Immunol. 2000; 22: 613-623.
4. Shigidi MM, Hashim RA, Idris MN, et al. Parasite diversity in adult patients with cerebral malaria a hospital-based, case control Study. Am J Trop Med Hyg. 2004; 71: 754-757.
5. Pérignon JL, Druilhe P. Immune mechanisms underlying the premunition against Plasmodium falciparum malaria. Mem Inst Oswaldo Cruz 1994; 89: 51-53.
6. Bouaharoun Tayoun H, Attanath P, Sabchrareon A, et al. Antobodies that protect humains against Plasmodium falciparum blood Stage do not their own inhibit parasite growth and invasion in vitro but act in cooperation with monocytes J. Exp Med. 1990; 172: 1633-1641.
7. Cohen S, MC Gtrgot IA, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature 1961; 192: 733-737.
8. Djibangar TA. Pofil des cellules cytotoxiques NK et TCD8 aux cours des différentes formes du paludisme Côte d'Ivoire. Master d'immunologie et infection. Dakar Université Cheikh Anta Diop de Dakar Faculté de Médecine, de Pharmacie et d'Odontologie. 2015; 334.
9. Ferlazzo G, Munz C. NK compartments and their activation by dendritic cells. J immunol. 2004; 172: 1333-1339.
10. Rakotoarivelo RA, Raveloson HF, Andrianasolo R, et al. Aspects cliniques et thérapeutiques du paludisme grave de l'adulte en milieu hospitalier à Antananarivo, Madagascar. Bull Soc Pathol Exot. 2009; 102: 215-216.
11. Hamala Sissoko Cheick. Paludisme sévère et compliqué chez l'adulte courant l'année 2003 au service de réanimation de l'hôpital Gabriel Touré. Thèse de doctorat en Médecine et Odonto-stomatologie. Mali Université de Bamako. Faculté de Médecine. 2006.
12. Coulibaly CK. Evaluation du rôle de la cellule NK Natural Killer dans le paludisme grave du sujet adulte vivant en zone endémique Côte d'ivoire. These de doctorat en medecine. Abidjan UFR Sciences Médicales Abidjan. 2016. N°6087.
13. Mohan K, Stevenson MM. Acquired immunity to asexual blood stages in Sherman IW Ed Malaria Parasite Biology Pathogenesis and Protection. Washington, ASM Press. 1998; 467-493.
14. Ribeiro-Dias F, Tosta CE. Dynamics and kinetics of natural killer cell cytotoxicity in human malaria as evaluated by a novel stepwise cytotoxicity assay. Rev Soc Bras Med Trop.

2006; 39: 357-364.
15. Dogruman-Al F, Fidan I, Kustimur S, et al. Determination of the expression of lymphocyte surface markers and cytokine levels in a mouse model of Plasmodium berghe. New Microbiol. 2009; 32: 285-291.
16. www.afrikibouge.com/pubilcations/
17. Agudelo O, Bueno J, Villa A, et al. High IFN-gamma and TNF production by peripheral NK cells of Colombian patients with different clinical presentation of Plasmodium falciparum. Malaria Journal. 2012; 11: 38.
18. Mavoungou E, Luty AJ, Kremsner PG. Natural Killer NK cell mediated cytolysis of PF infected Human red blood cell in vitro. Eur cytokine Netw. 2003; 14: 134-142.
19. Roetynck S, Baratin M, Vivier E, et al. Cellules natural Killer et immunité innée contre le paludisme. Med Sci Paris. 2006; 22: 739-744.
20. Riley EM, Ousman J, Whittle HC. CD8+ T-Cells inhibit Plasmodium falciparum lymphoproliferation and gamma interferon production in cell preparations from some malaria immune individuals Infect. Immun. 1989; 57: 1281.