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SHORT REPORT: DIFFERENTIAL EVOLUTION OF IMMUNOGLOBULIN G1/G3 ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* $MSP1_{19}$ OVER TIME IN MALARIA-IMMUNE ADULT SENEGALESE PATIENTS

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Abstract. This study examined the evolution of immunoglobulin (Ig) G1 and IgG3 antibodies against the asexual stage *Plasmodium falciparum* protein, $MSP1_{19}$, before and after a heavy malaria transmission period in clinically immune Senegalese subjects living under different epidemiological conditions. Plasma was tested for antibodies to a yeast-produced, recombinant $PfMSP1_{19}$ antigen (the Q-KNG allelic variant) that has previously been demonstrated to react with IgG1, IgG3, or both in the majority of these people. Anti-*P. falciparum* antibodies of the IgG1 and IgG3 subclasses, previously reported to be associated with protection, were shown to evolve independently one from another after the transmission period in both settings. These results suggest differential regulation of $MSP1_{19}$-specific IgG1 and IgG3. The precise role of these antibody isotypes in maintaining malaria immunity remains to be determined.

Protection against asexual blood stage *Plasmodium falciparum* has been reported to involve cytophilic type immunoglobulin (Ig) isotypes IgG1 and IgG3. Isotypes IgG2 and IgG4 have been proposed to antagonize the development of protection[1]; IgG3-dependent protection has been reported to be associated with recognition of the merozoite surface proteins MSP2 and MSP3. Protection related to IgG1 has been reported to be associated with reactivity with the 19-kDa C-terminus of MSP1 ($MSP1_{19}$).[2–4] However, there is no clear understanding of how or whether antibody subclass-dependent (Ab) protection from malaria is maintained in endemic areas. To address this issue, we measured IgG1 and IgG3 subclasses of antibodies that react with the $MSP1_{19}$ vaccine candidate antigen (Ag) over time. Specifically, antibody isotypes were determined before and after the highest transmission period (HTP) in clinically immune populations exposed regularly to *P. falciparum.*

Adults from the villages of Dielmo (n = 38) and Ndiop (n = 35) in Senegal were enrolled into this study. The characteristics of this patient population have been described previously.[5–7] All patients provided informed consent and this study was approved by the ad hoc ethical committee of the Institut Pasteur in Dakar under the auspices of national Senegalese authorities. It is of note that most patients had IgG and/or IgM to blood stage Ags. Fifty percent (in Dielmo) or 70% (in Ndiop) had anti-$MSP1_{19}$ IgG Abs, mostly restricted to IgG1 and IgG3 isotypes with virtually no IgG2 and IgG4.[7] Details of the epidemics, the periods of malaria transmission, and the sampling schedule are shown in Figure 1. Blood sampling and processing was performed exactly as previously described.[7] The present study covers the 1996–1997 period. Only villagers with no detectable blood parasites by means of the Quantitative Buffy Coat® test (Becton-Dickinson, Abijan, Ivory Coast) were included in the study.

Plasmodium falciparum $MSP1_{19}$ was obtained and secreted recombinant proteins ($yPfMSP1_{19}$) from *Saccharomyces cerevisiae* and purified as described.[8] Four analogs corresponding to amino-acid variations in the first (Q versus E) and the second (KNG versus TSR) EGF-like motifs of $MSP1_{19}$ were obtained using similar procedures and they were fused to a 6-histidine tag that is not immunogenic. The protein used in this study was the Q-KNG analog. This protein was recognized by IgG1 and IgG3 Abs, in contrast to other analogs that differed significantly in IgG3 Ab detection.[9]

The enzyme-linked immunosorbent assay determination of specific IgG1/IgG3 was performed as previously described.[7] Mouse anti-human IgG1 and IgG3 Abs (diluted 1/15,000) were purchased from Bionostics (Bionostics, Wyboston Beds, UK). To minimize plate-to-plate and day-to-day variations, data were recorded as optical density (OD) ratios over negative control (a pool of European plasma), and OD ratios > 1.5 were considered positive.[10] Fisher's exact test was used to compare groups (chi-square test).

To compare the evolution over time of specific IgG1/IgG3, we categorized isotype patterns in 4 classes: $IgG1^-$ $IgG3^-$ (negative), $IgG1^+$ $IgG3^-$, $IgG1^-$ $IgG3^+$, and $IgG1^+$

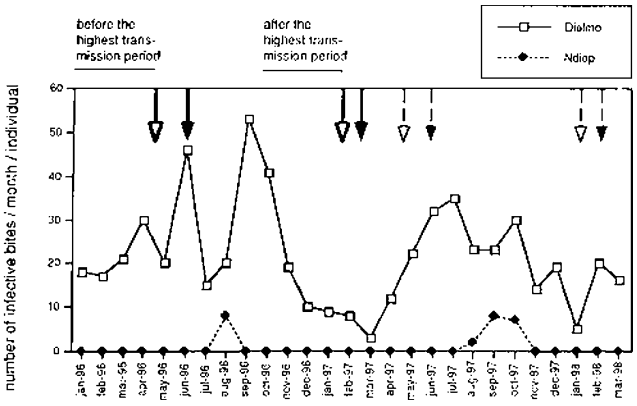

FIGURE 1. Malaria epidemics in the villages of Dielmo **(open squares)** and Ndiop **(closed diamonds)** and sampling schedule. Indicated are the numbers of infective bites per month and per individual (y-axis) and the month by month records over 2 consecutive years (x-axis). On the top of the figure, the **arrows** indicate the period of sampling (**white arrow**: Dielmo; **black arrow**: Ndiop). Also indicated are the periods before and after the high transmission periods when samples were obtained. **Plain arrows** indicate the period corresponding to the present study; **dashed arrows** indicate a later study.

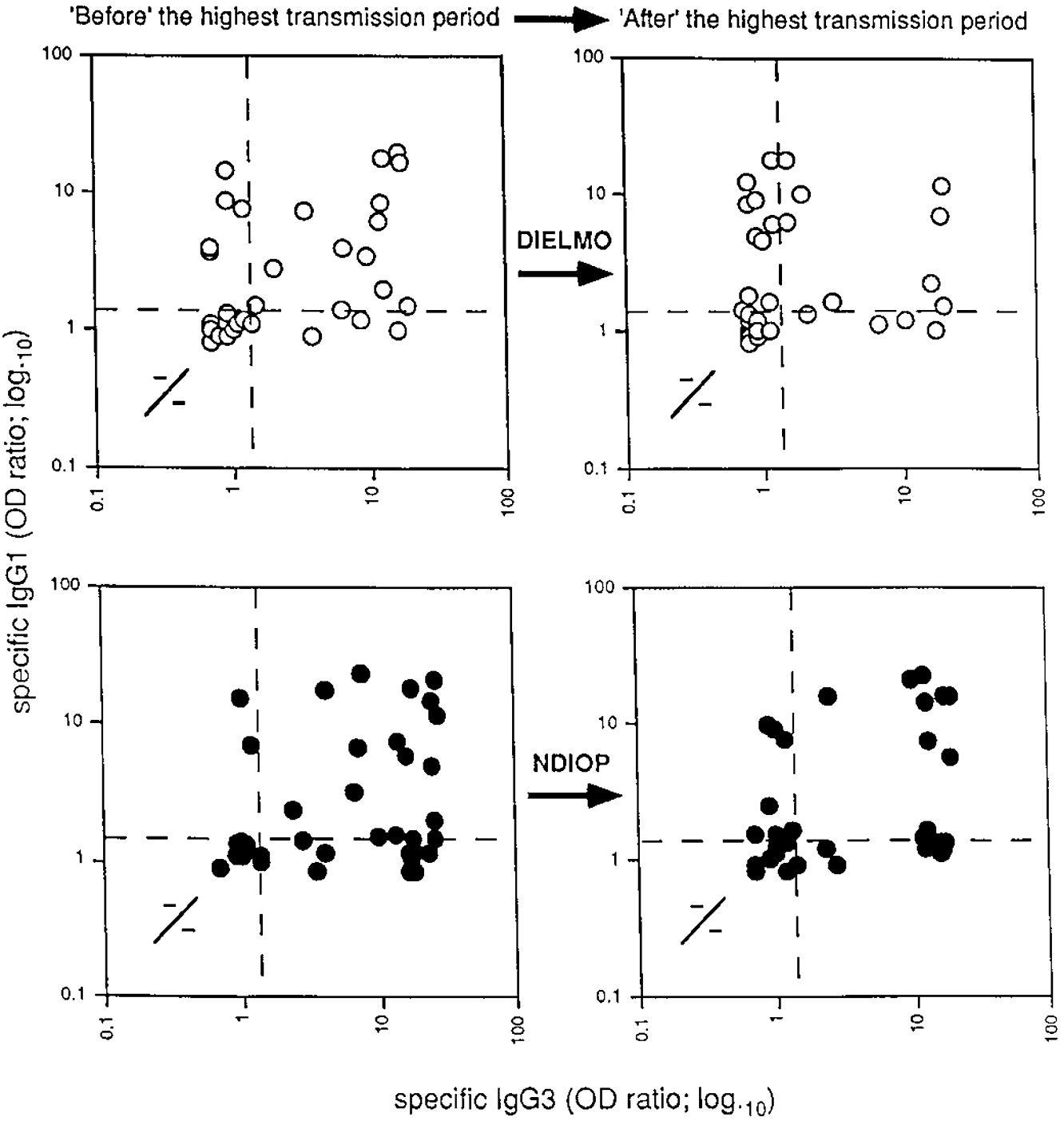

FIGURE 2. Profiles of IgG1- versus IgG3-specific antibody responses to yPfMSP1$_{19}$-Q-KNG in Dielmo and Ndiop over time. Plotted are OD ratios for IgG1- (y-axis) and IgG3- (x-axis) specific antibodies detected in the same individual's plasma sample in the Dielmo **(top panels)** and Ndiop **(bottom panels)** donors. The right panels represent individual values before the highest transmission period (April 1996 in Dielmo; May 1996 in Ndiop). The left panels represent individual values after the high transmission period (mid-January 1997 in Dielmo; mid-February 1997 in Ndiop). Individual data in Dielmo are expressed as **open circles** and in Ndiop as **closed circles.** Data are expressed as OD ratios on a $\log_{10}$ scale. Dashed lines indicate values for positive thresholds (1.5 times the control) which define 4 quadrants: IgG1$^-$ IgG3$^-$ (negative; **bottom left**), IgG1$^+$ IgG3$^-$ **(top left),** IgG1$^+$ IgG3$^+$ **(top right),** and IgG1$^-$ IgG3$^+$ **(bottom right).**

IgG3$^+$. There was a significant decrease of IgG1$^+$ IgG3$^+$ in both settings after the HTP (Figure 2; chi-square = 3.96, $P < 0.04$ in Dielmo; chi-square = 5.21, $P < 0.02$ in Ndiop). There was a significant increase of IgG1$^+$ IgG3$^-$ samples in Dielmo (chi-square = 6.25, $P < 0.01$; Figure 2, top panels) and in Ndiop (chi-square = 4.98, $P = 0.02$; Figure 2, bottom panels). We then determined whether the presence of Abs of one subclass was associated with the presence of Abs of the other subclass. Such an association was found in both villages before the HTP (Dielmo: chi-square = 9.8, $P = 0.003$; Ndiop: chi-square = 5.3, $P = 0.04$), but not after this period.

Data presented in this study show that anti-MSP1$_{19}$ IgG1

and IgG3 evolve independently one from another after the HTP. These data are emphasized by independent isotype reactivity patterns occurring in populations living under different conditions. This study was repeated in 1997–1998 (dashed arrows in Figure 1), although in a smaller number of people, especially in Ndiop; similarly, data were obtained in Dielmo (data not shown). The IgG1 and IgG3 Ab responses are generally considered together in most *P. falciparum*–related immunological studies. To our knowledge, this study is the first that indicates that specific IgG1 and IgG3 responses are regulated before and after the seasonal parasite exposure or reexposure in endemic areas. These data raise the possibility that the number of infective bites affects such regulation in people who have acquired a protective immunity in holoendemic areas (such as Dielmo) and in mesoendemic areas (such as Ndiop).

The half-life for IgG1 is ~ 3 weeks; for IgG3, it is 1 week. It is likely that there is a sustained and rapid renewal of specific IgG1 and IgG3, even in Ndiop, where transmission is seasonal, but probably not absent during the so-called dry season, because many adults have specific IgM.[7,11,12] These data, along with results obtained for this and other protective *P. falciparum* Ags,[12] argue that the production of specific IgG1 and IgG3 is adaptive. It would be of interest to investigate further the respective roles of long-lived IgG1 versus short-lived IgG3, not only in the acquisition but also in the maintenance of immunity to *P. falciparum* blood stage in populations exposed to this parasite.

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REFERENCES

1. Bouharoun-Tayoun H, Druilhe P, 1992. *Plasmodium falciparum* malaria: evidence for isotype imbalance which may be responsible for delayed acquisition of protective immunity. *Infect Immun 60:* 1473–1481.
2. Rzepczyk CM, Hale K, Woodroffe N, Bobogare A, Csurhes P, Ishii A, Ferrante A, 1997. Humoral immune responses of Solomon islanders to the merozoite surface antigen 2 of *Plasmodium falciparum* show pronounced skewing toward antibodies of the immunoglobulin G3 subclass. *Infect Immun 65:* 1098–1100.
3. Oeuvray C, Bouharoun-Tayoun H, Gras-Mass H, Bottius E, Kaidoh T, Aikawa M, Filguera MC, Tartar A, Druilhe P, 1994. Merozoite surface protein-3: a malaria protein inducing antibodies that promote *Plasmodium falciparum* killing by cooperation with blood monocytes. *Blood 84:* 1594–1602.
4. Egan AF, Morris J, Barnish G, Allen S, Greenwood BM, Kaslow DC, Holder AA, Riley ER, 1996. Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. *J Infect Dis 173:* 765–769.
5. Trape JF, Rogier C, Konate L, Diagne N, Bouganali H, Canque B, Legros F, Badji A, Ndiaye A, Ndiaye A, Brahimi K, Faye O, Druilhe P, Pereira da Silva L, 1994. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity in a community living in a holoendemic area of Senegal. *Am J Trop Med Hyg 51:* 123–137.
6. Trape JF, Rogier C, 1996. Combating malaria morbidity and mortality by reducing transmission. *Parasitol Today 12:* 236–240.
7. Nguer CM, Diallo TO, Diouf A, Tall A, Dièye A, Perraut R, Garraud O, 1997. *Plasmodium falciparum*—and merozoite-surface protein 1—specific antibody isotype balance in immune Senegalese adults. *Infect Immun 65:* 4873–4876.
8. Kaslow DC, Hui G, Kumar S, 1994. Expression and antigenicity of *Plasmodium falciparum* major merozoite surface protein-1 (MSP1$_{19}$) variants secreted from *Saccharomyces cerevisiae*. *Mol Biochem Parasitol 63:* 283–289.
9. Diallo TO, Spiegel A, Diouf A, Perraut R, Kaslow DC, Garraud O, 2001. IgG1/IgG3 antibody responses to analogs of recombinant yPfMSP1$_{19}$: a study in immune adults living in areas of *P. falciparum* transmission. *Am J Trop Med Hyg 64:* 204–206.
10. Garraud O, Nkenfou C, Bradley JE, Perler FB, Nutman TB, 1995. Identification of recombinant filarial proteins capable of inducing polyclonal and antigen-specific IgE and IgG4 antibodies. *J Immunol 155:* 1316–1325.
11. Diallo TO, Nguer CM, Dieye A, Spiegel A, Perraut R, Garraud O, 1999. Immune responses to *P. falciparum* MSP1 antigen: lack of correlation between antibody responses and the capacity of peripheral cellular immune effectors to respond to this antigen in vitro. *Immunol Lett 67:* 217–221.
12. Perraut R, Mercereau-Puijalon O, Diouf B, Tall A, Guillotte M, Le Scanf C, Trape JF, Spiegel A, Garraud O, 2000. Season-dependent fluctuation of antibody levels to *P. falciparum* parasitized red blood cell associated antigens in two Senegalese villages with different transmission conditions. *Am J Trop Med Hyg 62:* 746–751.