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Multiple sclerosis: peripheral mononuclear cells inhibit *Plasmodium falciparum* growth and are activated by parasite antigens

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The human genome has been subjected to selective pressures to resist to infectious agents in spite of a heavy segregational load. With this regard, thalassaemia and glucose-6-phosphate dehydrogenase deficiency have been considered an efficient genetic protection against *P. falciparum* malaria in Sardinia, insular Italy[1,2]. In this island, some multiple sclerosis (MS)-associated HLA haplotypes have the highest odds ratios in the same highest-rate malarious areas of the island[3]. Moreover, tumor necrosis factor (TNF) polymorphisms epidemiologically associated with both MS and malaria are ten-fold more frequent amongst Sardinians compared to other populations worldwide[4]. A possible association between MS and malaria in this island was never analysed experimentally. We studied the immunological response of mononuclear cells to *P. falciparum* and the killing effect of macrophages on parasites in Sardinian MS patients and in matched healthy controls (HC).

Altogether 12 Sardinian MS patients (mean age 29 ± 5 years) and 12 matched healthy controls of same ethnicity were enrolled in this study. All individuals gave written consent to participate in the study. Parasitised erythrocytes were lysed with 0.05% saponin PBS and pellet sonicated six times on ice with Bandelin Sonopuls. A mixture of *P. falciparum* antigens was obtained; 2×10^5 mononuclear cell (MNC)/well from MS and HC were plated in duplicate on a microtiter plate alone or added with either 10 μg/ml lipopolysaccharide (LPS) or *P. falciparum* lysate (1 *Plasmodium*/MNC). After 48 h, a stimulation index (SI) was calculated as the unit of proliferation measurement (SI = Mean count of duplicate cultures with LPS/Mean counts of duplicated cultures without LPS x 100) (Fig. 1). Compared to the background level of proliferation of the untreated MNC (assuming it as 100%) there was a highly significant difference of the anti-*P. falciparum* proliferative response, but not of the LPS-driven response between the two groups: 158.3 ± 23% in MS and 121.7 ± 19.5% in HC, respectively ($p = 0.01$; Fig. 1).

We also performed a *P. falciparum* inhibition test in the presence of macrophages from MS and HC (Fig. 2). The macrophage containing adherent fraction of MNC was identified by flow cytometry and dispensed in eight replicates on a microtiter plate added with *P. falciparum* cultures. Parasite growth was determined by measuring its lactate dehydrogenase activity (pLDH)[5] with a spectrophotometer at 650 nm. *P. falciparum* growth was inhibited by 8.8 ± 6.5% with

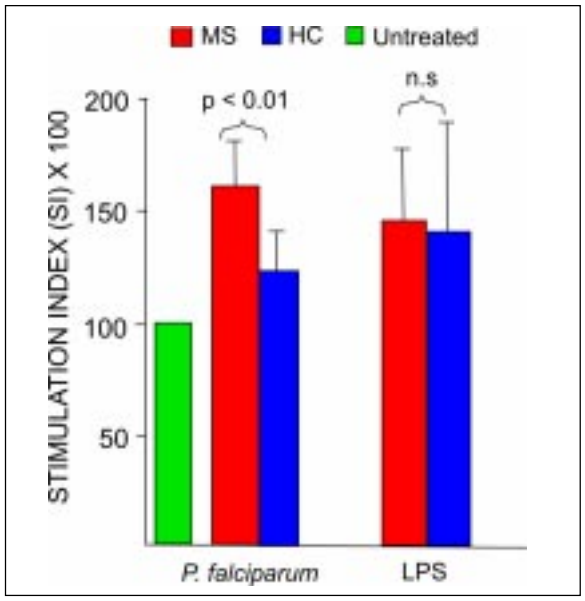

Fig. 1: Stimulation index of non-stimulated (untreated, green bar, assuming it as 100%), LPS-stimulated and *P. falciparum*-stimulated peripheral blood mononuclear cells (MNC) from 12 Sardinian MS patients (MS, red bars) and 12 Sardinian healthy donors (HC, blue bars); LPS and *P. falciparum* stimulated MNC proliferate enormously as compared to their relative untreated MNC (see text); *P. falciparum*-driven proliferation of MS MNC is significantly higher as compared to HC ($p < 0.01$)

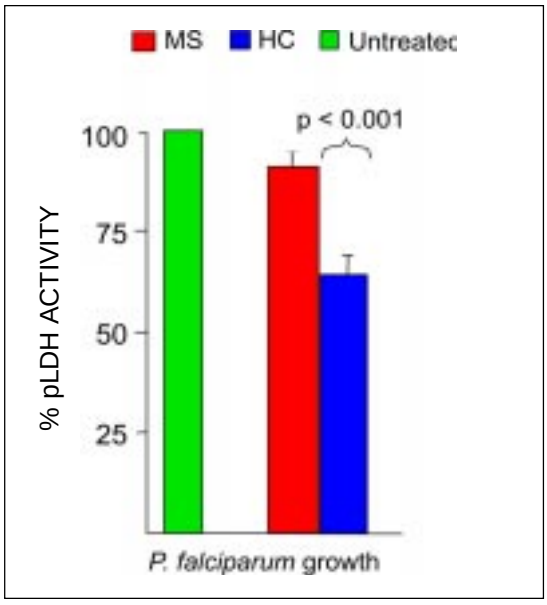

Fig. 2: Proportion of pLDH activity of *P. falciparum* in three different conditions: untreated (green bar, 100% activity), treated with macrophages from HC (blue bar, 12 subjects) and from MS (red bar, 12 subjects). As compared to the untreated condition there is a significant reduction of *P. falciparum* growth when using MS-derived macrophages as compared to HC-derived macrophages ($p < 0.001$)

macrophages from HC and by 36 ± 7.2% with macrophages from MS patients ($p = 0.001$; Fig. 2). In this study we report that *P. falciparum*, but not LPS-driven proliferative MNC response was significantly elevated in MS compared to controls of same ethnicity. These data indicate that Sardinian individuals affected by MS have a stronger anti-*P. falciparum* response than healthy controls. To our knowledge this is the first report of such an association, suggesting the possibility that malaria may have selected genetic traits in Sardinia. Perhaps not coincidentally and not justified by improved diagnosis[6], a 3-fold increase of MS incidence subsequently occurred beginning from 1950[7,8]. In 1951, the WHO-coordinated campaign eradicated malaria from Sardinia. This increased incidence over time still remains an unsolved enigma but, being a 50-year span too short for a genetic change, an environmental change is a likely reason for the increased risk. Our experimental evidences indicate that some patterns of macrophage response formerly positively selected against severe forms of *P. falciparum* malaria, may now increase the susceptibility toward abnormal immune-mediated conditions, such as MS.

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