Inhibition of early mouse plasmacytoma development after *Plasmodium* infection

Highlights

- *Plasmodium* infection inhibited early mouse plasmacytoma development;
- This effect was mediated by ASGM1+ immune cells;
- It was correlated with an overproduction of IL-12, TNF and IFN-γ

Abstract:

Background: Inhibition of early cancer development, through enhancement of cancer immunosurveillance by innate cells has been reported after infection with different microorganisms. Since *Plasmodium* infection modulates the innate immune system, we examined the effect of *Plasmodium* infection on cancer immunosurveillance.

Methods: As a model, we used *Plasmodium yoeli 265 BY* infection of BALB/c mice and administration of TEPC.1033.C2 plasmacytoma cells.

Results: *Plasmodium* infection effectively inhibited the early development of plasmacytoma. The protective effect of infection was not due to a direct cytopathic destruction of cancer cells by the parasite. *Plasmodium* infection induced the production of proinflammatory cytokines such as interleukin-12 and interferon-gamma, known to be involved in cancer immunosurveillance. Depletion of $ASGM1^{+}$ cells *in vivo* largely suppressed the protective effect of the parasite.

Conclusions: This observation suggest *Plasmodium* infections in humans might participate to the low incidence of multiple myeloma in countries where malaria is frequent and should be taken into account in public health policy of these countries.

Keywords: *Plasmodium yoeli*; plasmacytoma ; interferon-gamma; interleukin-12; ASGM1+ cells

Abbreviations: NK: Natural Killer; IFN: Interferon; IL: interleukin; TNF : Tumor necrosis factor; LDV: Lactate dehydrogenase-elevating virus; TLR: Toll-like receptor; ASGM1: asialoganglioside-GM1;

1. Introduction

Efficient control of early cancer development depends on immunosurveillance mediated by immune cells including Natural Killer (NK) and NKT cells and by their production of cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor (TNF) [1, 2]. Although many cancers may be caused by infectious agents, some infections may enhance cancer immunosurveillance, leading to inhibition of early cancer development [3, 4]. In mouse experimental models, infections with lactate dehydrogenase-elevating virus (LDV) or *Trypanosoma brucei*, and stimulation with Toll-like receptor (TLR) ligands similarly decrease early growth of tumors such as plasmacytoma/myeloma and mesothelioma [5-8]. This early inhibition of cancer development requires asialoganglioside-GM1 (ASGM1) positive cells and the production of proinflammatory cytokines such as IFN-γ and interleukin-12 (IL-12).

Multiple myeloma incidence is lower in developing countries where infections with a large array of viruses, bacteria and parasites, including *Plasmodium*, are higher than in industrialized countries [9]. Interestingly, similarly to LDV, *Plasmodium* infection results in activation of NK and NKT cells, and in increased IFN-γ production [10]. Therefore, we investigated whether mouse *Plasmodium* infection could also result in early inhibition of plasmacytoma development.

2. Materials and Methods

2.1. Mice

Female BALB/c mice were bred at the Ludwig Institute for Cancer Research by P. Gomez Pinilla and used at the age of 7–10 weeks. Experiments were approved by the Comité d'Ethique facultaire pour l'Expérimentation Animale - Secteur des Sciences de la Santé - Université catholique de Louvain (ref. 2018/UCL/MD/007).

2.2. Plasmodium infection

Blood-stage samples of *Plasmodium yoeli 265 BY* were kindly given by S. Pied (Lille, France). The *Plasmodium* stock was checked to be free of LDV contamination. Mice were infected by i.p. injection of 10^6 infected erythrocytes (iRBCs).

2.3. Tumor cells

TEPC.1033.C2 plasmacytoma cell line, initially received from M. Potter, was cultured in supplemented Iscove's Modified Dulbecco's Medium [8].

2.4. Antibodies

Cell depletion with anti-asialoganglioside-GM1 (ASGM1) polyclonal antibody was performed as described previously [8].

2.5. Cytokine assays

Cytokines were measured in sera by ELISA. IL-12 levels were measured as described previously [11]. For TNF and IFN-γ, Maxisorb ELISA plates (Nunc, UK) were coated with 4 µg/mL of anti- TNF-α (ref. 14-7325-85) and 2 µg/mL of anti-mouse IFN-γ (ref. 14-7311-85, eBioscience Inc.), respectively. After blocking in PBS with 10% FCS, samples were incubated for 2hr at 37°C, followed by detection antibodies (4 µg/mL of biotinylated TNF-a Antibody Cocktail (ref .13-7326-85) and 4 µg/mL of biotinylated IFN-g Monoclonal Antibody (ref. 13-7311-85, eBioscience Inc.), by avidin-HRP (1:2000 dilution; ref. 405103, Biolegend), by 1-Step™ Ultra TMB-ELISA Solution (ref. 34028, Thermo Fisher Scientific), and by 20 µl of stop solution (2M H_2SO_4). The absorbance reads were made at 450 nm, using a 96-well plate spectrophotometer (VERSAmax, Molecular Device).

2.6. Statistical analysis

Statistical analysis was performed with Prism 6 (GraphPad Software, La Jolla, CA) using a non-parametric test (Mann–Whitney), and a Log-rank test (survival curve).

3. Results

3.1. Effect of Plasmodium yoeli 265 BY infection on plasmacytoma development and on cytokine production.

The impact of *Plasmodium yoeli* infection on early plasmacytoma growth was examined by administration of TEPC.1033.C2 cells into control BALB/c mice or into animals that had been infected 10 days earlier. The majority of uninfected mice quickly developed tumors and died around 20 days after tumor cell administration (Figure 1A). In contrast, plasmacytoma development in mice infected with *Plasmodium* was significantly delayed (Figure 1A, $p<0.0001$, three pooled independent experiments). Although most animals finally developed tumor and died, some of them were still free of clinical signs of tumor for more than six weeks. To determine whether the alteration of plasmacytoma development after *Plasmodium* infection was caused by direct effect of the parasite on cancer cells, we cultured TEPC.1033.C2 cells in the presence of *Plasmodium*. *In vitro* tumor cell replication was measured on day 3, 5, and 7. No difference in cell proliferation was found between with and without *Plasmodium* (Figure 1B, representative of two independent experiments).

Activation of immune innate cells involved in cancer immunosurveillance results in production of proinflammatory cytokines. After *Plasmodium* infection, we observed a significant increase of IL-12 (Figure 1C, $p=0.0294$), and a non significant increase of IFN-γ ($p=0.0797$) and of TNF ($p=0.2676$).

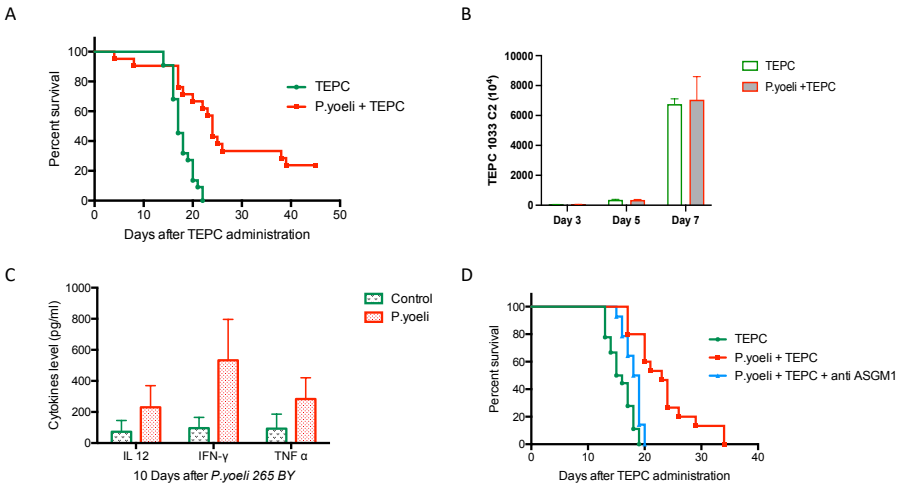

Figure 1

Figure 1. Effect of *Plasmodium* infection on TEPC.1033.C2 plasmacytoma cell early growth. A. Survival of BALB/c mice uninfected or infected with *Plasmodium yoeli 265 BY* 10 days before administration of $4x10^4$ TEPC.1033.C2 cells. Pooled data from three independent experiments, 22 mice per group. B. Cell number after different times of culture of $4x10^4$ TEPC.1033.C2 cells in the presence of $2x10^6$ iRBCs. Results as means ± SEM for triplicate cultures, representative of two independent experiments. C. IL-12, IFN-γ and TNF were measured in sera of BALB/c mice uninfected or infected for 10 days with *Plasmodium yoeli 265 BY*. Results as means ± SEM for 8 mice per group from two independent experiments. D. Survival of BALB/c mice uninfected or infected with *Plasmodium yoeli 265 BY* 10 days before administration of $4x10^4$ TEPC.1033.C2 tumor cells, without or with anti-ASGM1 treatment. Pooled data from three independent experiments, 18 mice per group.

3.2. Involvement of ASGM1+ cells in the protective effect of Plasmodium infection on early plasmacytoma growth.

ASGM1+ cells have been found previously to be responsible for the protective effect of LDV and of TLR ligands on early cancer growth [5, 7, 8]. To determine whether these cells were also involved in the protective effect of *Plasmodium*, mice were treated with anti-ASGM1 polyclonal antibody two days before, and the day of tumor cell inoculation. As shown in Figure 1D, this treatment completely suppressed the protective effect of *Plasmodium* protection ($p=0.0002$ for TEPC + *P. yoeli* versus TEPC + *P. yoeli* + anti-ASGM1, three pooled independent experiments), demonstrating the role of ASGM1+ cells.

4. Discussion

Although the oncogenic capacity of many infectious agents has been demonstrated, some of them have been reported to enhance cancer immunosurveillance, both in humans and in animal models [3-8]. Moreover, *Plasmodium* infection occurring at the time or after cancer cell inoculation in mouse models of Lewis lung cancer, breast cancer, and hepatoma inhibits tumor development through enhancement of antitumoral immune responses [12-14]. These observations have led to the approval of clinical trials of malaria immunotherapy in China. In our study, we found that the enhancement of innate immune responses after *Plasmodium* infection may also result in a more efficient cancer immunosurveillance, leading to further prevention of plasmacytoma growth. This preventive effect of *Plasmodium*, as well as of LDV infection [5, 7] is mediated by ASGM1+ cells that include NK cells, NKT cells and a subpopulation of CD8+ T cells that share the capacity of early non cognate response, including IFN-γ production [15]. A similar enhancement of cancer immunosurveillance has been reported after ligation of TLR receptors, and especially of TLR9 [8]. Interestingly, *Plasmodium* parasites activate the innate immune system through ligation of TLR9 by their byproduct hemozoin [16],

and therefore this mechanism might be at least partly responsible for the parasite preventive effect.

IL-12, produced by dendritic cells and macrophages, and activating NK and NKT cells, and IFN-γ, produced in response to IL-12 by those cells are strongly involved in antitumoral responses and have been shown to be involved in the preventive effect of infections on tumor growth [5-8, 13]. Although their production was enhanced after *Plasmodium* infection, we were not able to demonstrate their role in the prevention of plasmacytoma growth in our model, which may suggest that other mechanisms could also be involved.

Whatever the molecular mechanisms responsible for the protective effect of *Plasmodium* infection on plasmacytoma early growth, our results strongly suggest that common infections may have an incidence on the prevalence of cancers that are sensitive to immunosurveillance. This effect may be to decrease this incidence or to enhance it. Indeed, infectious agents that can inhibit NK cell activation and IFN-γ production, such as *Schistosoma*, rather enhance the early growth of plasmacytoma in mice and might increase the incidence of multiple myeloma in exposed populations (M. Mandour, manuscript in preparation). It may nevertheless be suggested that in low and middle income countries, the global effect of infections enhancing cancer immunosurveillance exceeds factors that promote tumor growth, resulting in a global lower incidence of some cancers, including multiple myeloma [9].

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