Cytokine 95 (2017) 102–112

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Early serum biomarker networks in infants with distinct retinochoroidal lesion status of congenital toxoplasmosis

Thádia Evelyn de Araújo [a,b], Jordana Grazziela Coelho-dos-Reis [b], Samantha Ribeiro Béla [b], Ana Carolina Aguiar Vasconcelos Carneiro [c], Anderson Silva Machado [c], Ludmila Melo Cardoso [b], Ágata Lopes Ribeiro [b], Michelle Hallais França Dias [b], Gláucia Manzan Queiroz Andrade [d], Daniel Vitor Vasconcelos-Santos [e], José Nélio Januário [f], Andréa Teixeira-Carvalho [b], Ricardo Wagner Almeida Vitor [c], Eloisa Amália Vieira Ferro [a], Olindo Assis Martins-Filho [a,b,*], on behalf of the UFMG Congenital Toxoplasmosis Brazilian Group – UFMG-CTBG

[a] Universidade Federal de Uberlândia, Avenida João Naves de Ávila 2121, Santa Mônica, 38408-100 Uberlândia, MG, Brazil
[b] Grupo Integrado de Pesquisas em Biomarcadores, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Avenida Augusto de Lima, 1715 Barro Preto, 30190-002 Belo Horizonte, MG, Brazil
[c] Departamento de Parasitologia, Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brazil
[d] Departamento de Pediatria, Universidade Federal de Minas Gerais, Avenida Professor Alfredo Balena 190, Santa Efigênia, 30130-100 Belo Horizonte, MG, Brazil
[e] Departamento de Oftalmologia e Otorrinolaringologia, Faculdade de Medicina da UFMG, Belo Horizonte, MG, Brazil
[f] Núcleo de Ações e Pesquisa em Apoio Diagnóstico (NUPAD), Universidade Federal de Minas Gerais, Avenida Professor Alfredo Balena 190, Santa Efigênia, 30130-100 Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 6 February 2017
Accepted 17 February 2017

Keywords:
Congenital toxoplasmosis
Retinochoroiditis
Infants
Biomarkers

ABSTRACT

The present study characterized the early changes in the serum chemokines/cytokine signatures and networks in infants with congenital-toxoplasmosis/(TOXO) as compared to non-infected-controls/(NI). TOXO were subgrouped according to the retinochoroidal lesion status as no-lesion/(NL), active-lesion/(ARL), active/cicatricial-lesion/(ACRL) and cicatricial-lesion/(CRL). The results showed that TOXO display prominent chemokine production mediated by IL-8/CXCL8, MIG/CXCL9, IP-10/CXCL10 and RANTES/CCL5. Additionally, TOXO is accompanied by mixed proinflammatory/regulatory cytokine pattern mediated by IL-6, IFN-γ, IL-4, IL-5 and IL-10. While TNF appears as a putative biomarker for NL and IFN-γ/IL-5 as immunological features for ARL, IL-10 emerges as a relevant mediator in ACRL/CRL. IL-8/CXCL8 and IP-10/CXCL10 are broad-spectrum indicators of ocular disease, whereas TNF is a NL biomarker, IFN-γ and MIG/CXCL9 point out to ARL; and IL-10 is highlighted as a genuine serum biomarker of ACRL/CRL. The network analysis demonstrated a broad chemokine/cytokine crosstalk with divergences in the molecular signatures in patients with different ocular lesions during congenital toxoplasmosis.

1. Introduction

The *Toxoplasma gondii* is a protozoan parasite of global distribution capable of infecting great part of the warm-blooded animals. *T. gondii* infects approximately one third of the world population and endemic areas present extremely high seroprevalence for this parasite [1–3]. The majority of the cases remain asymptomatic despite the parasite's versatility and wide dissemination [4]. However, the infection by *T. gondii* can be specially damaging during the infection in pregnancy, which may result in vertical transmission of toxoplasmosis. Congenital toxoplasmosis is, by far, the most significant and severe form of the disease [5,6].

Studies conducted in endemic areas of Brazil indicate the highest prevalence of the congenital toxoplasmosis in comparison with the rest of the world [7,3]. In 80% of the cases, the newborns present retinochoroiditis at the time of birth, 50% of which manifest predominantly the severe forms of retinochoroidal lesion. These alarming findings among infants with congenital toxoplasmosis from Brazil reveal increased severity of ocular disease as compared to other clinical ocular findings reported around the globe [8].

* Corresponding author at: Grupo Integrado de Pesquisas em Biomarcadores, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Avenida Augusto de Lima, 1715 Barro Preto, Belo Horizonte, Minas Gerais 30190-002, Brazil.
E-mail addresses: oamfilho@cpqrr.fiocruz.br, oassismartins@gmail.com (O.A. Martins-Filho).

http://dx.doi.org/10.1016/j.cyto.2017.02.018
1043-4666/

Dimorphism in parasites and circulation of atypical strains could be one explanation as to why severity and prevalence of ocular lesions increase in endemic areas [9]. On the other hand, the outcome of the infection by the *T. gondii* relies on the interaction between complex inflammatory responses stimulated by the parasite as well as the *T. gondii*-triggered modulatory mechanisms, mainly in pregnant women [5]. The immunopathogenesis induced by the parasite is well known during the infection in adults [10], however, the data for ocular congenital toxoplasmosis in humans are still scarce.

Aiming at describing the profiles of innate and adaptive immune cell subsets on infants with ocular toxoplasmosis, recent studies have revealed distinct inflammatory and modulatory events occurring in children with different types of ocular lesions during congenital toxoplasmosis. Machado and collaborators (2014) [11] revealed selected expansion of $CD14^{+}CD16^{+}HLA\text{-}DR^{high}$ monocytes and $CD56^{dim}$ cytotoxic NK-cells during active retinochoroidal lesion, whereas relevant connections between NK and $CD8^{+}$ T-cells with a broad range of cell subsets are present during cicatricial retinochoroidal lesions. These results suggest communication between different cell subsets, but the mechanisms and molecules that mediate these interactions and induce expansion, activation and migration of such subsets still remain unclear.

In this regard, both the innate and the adaptive immunity have roles on the control of *T. gondii* dissemination within the adult host. TNF and IFN-γ promote early and late events associated to cytotoxicity against *T. gondii*-infected cells exerted by effector $CD8^{+}$ T-cells [12,13]. Nevertheless, limiting the damages caused by the parasite is also dependent upon the capacity of the host to restrain the exacerbated inflammatory response by the production of modulatory molecules such as IL-4 and IL-10 [13–15].

In this context, the role of chemokines is to recruit immune cells by chemotaxis and work in the host-parasite relationship [16]. In toxoplasmosis, it has been reported that IL-8/CXCL8 plays a critical role in the inflammatory process of acute toxoplasmic retinochoroiditis [17,18]. In parallel, IP-10/CXCL10 is also responsible for the maintenance of the T-cell responses and the control of *T. gondii* in the eye during chronic infection in mice [19]. In resistant BALB/c mice, the chemokines IP-10/CXCL10, RANTES /CCL5 and MIG/CXCL9 are predominantly induced during chronic infection in the brain, and their expression is IFN-γ-dependent [20].

Considering these findings, the purpose of this study is to characterize the inflammatory and modulatory cytokine profile as well as the chemokine pattern of infants with congenital toxoplasmosis from Minas Gerais (Brazil) in light of their clinical ocular lesion status, using biomarkers networks and signatures.

2. Material and methods

2.1. Study population

This is a cross-sectional investigation carried out as part of a broader investigation on neonatal screening for congenital toxoplasmosis conducted by the UFMG Congenital Toxoplasmosis Brazilian Group. A flowchart illustrating the neonatal compendium of experimental design is provided in Fig. 1. A non-probabilistic convenience sampling was performed to select infants with Positive/Indeterminate results for ∗∗*anti-T. gondii* IgM screening by Enzyme-Linked Immunosorbent Assay (Q-Preven® TOXO, Biomerieux, France) (n = 143). Serum samples obtained by venipucture at 30–45 days after birth were used for the biomarker quantitative analysis.

Cases of congenital toxoplasmosis (TOXO, n = 121) were considered when infants, at one year of age, presented positive results for *anti-T. gondii* IgG ELFA test (ELFAVIDAS® TOXO, Biomerieux, France). Infants with negative results at one year of age were classified as non-infected control group (NI, n = 22).

Infants with congenital toxoplasmosis underwent ophthalmological evaluation performed by one of us (DVVS). The clinical classification of retinochoroidal lesions was defined by fundoscopic analysis as previously described [8,13]. Ocular toxoplasmosis status was defined after diagnosis of retinochoroidal lesions, which were classified as wounds in the retina. Based on ocular toxoplasmosis status, TOXO group was sub-divided into 4 categories referred as: (i) infants without retinochoroidal lesions (NL, n = 29); (ii) infants presenting active retinochoroidal lesions (ARL, n = 11); (iii) infants with active/cicatricial retinochoroidal lesions (ACRL, n = 45) and (iv) infants with cicatricial retinochoroidal lesions (CRL, n = 36).

The research protocol of this cross-sectional investigation was approved by the local Ethics Committee of Federal University of Minas Gerais (Minas Gerais state, Brazil), protocol number 298/06 and all mothers of infants included in this work agreed and provided written informed consent.

Serum samples collected at 30–45 days after birth were stored at −80 °C and used for serum biomarker analyses by flow cytometric bead array.

2.2. Flow cytometric bead array (CBA)

The chemokines IL-8/CXCL8, MCP-1/CCL2, MIG/CXCL9, IP-10/CXCL10 and RANTES/CCL5 were also assessed in the serum of infants with congenital toxoplasmosis. These molecules were measured by Cytometric Bead Array (CHEMOKINE CBA Kit - BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. The cytokine levels of IL-1β, IL-6, TNF, IL-12, IFN-γ, IL-4, IL-5, IL-10 and IL-17A in serum samples from infants with congenital toxoplasmosis were tested using enhanced sensitivity Cytometric Bead Array (Flex enhanced sensitivity CBA Kits - BD Biosciences, San Jose, CA, USA). Flex sets were combined together in a one assay set up as described by the manufacturer's instructions. Data acquisition and analysis was performed by flow cytometry using BD FACSVerse flow cytometer (Becton Dickinson, La Jolla, CA, USA) using BD FACSuite™ software (Becton Dickinson, La Jolla, CA, USA) for acquisition and FCAP Array™ Software Version 3.0 (Becton Dickinson, La Jolla, CA, USA) for analysis. The results were expressed as pg/mL (chemokines) or fg/mL (cytokines), as assessed by the standard curve using the forth-logistic regression parameter. The limits of detection were IL-8/CXCL8 = 2.5 pg/mL, MCP-1/CCL2 = 0.2 pg/mL, MIG/CXCL9 = 2.7 pg/mL, IP-10/CXCL10 = 2.8 pg/mL, RANTES/CCL5 = 1.0 pg/mL, IL-1β = 274.35 fg/mL, IL-6 = 409.62 fg/mL, TNF = 144.62 fg/mL, IL-12 = 191.48 fg/mL, IFN-γ = 172.09 fg/mL, IL-4 = 238.34 fg/mL, IL-5 = 407.19 fg/mL, IL-10 = 152.70 fg/mL and IL-17A = 239.95 fg/mL. All patient samples were assayed in the same batch using the same standard curve to avoid inter-assay variability. Samples were considered undetermined when unfitted by the standard curve and, therefore, excluded from the analysis.

2.3. Data analysis and statistics

The level of cytokines and chemokines measured in the sera obtained from patients with toxoplasmosis were compared by non-parametric tests based on analysis of variance (anova), Kruskal–Wallis test followed by Dunn's multiple comparison test. The Graphpad Prism software version 5.0 (San Diego, CA, USA) was used for data analysis.

2.4. Biomarker signature analysis

In this data analysis, initially, the whole universe of data of each cytokine and chemokine was used to calculate the global median value used as the cut-off to classify infants as with "low" or "high" counts of a given biomarker, as described previously [21]. The cut-offs of 50% was used to categorize each infant as presenting "low"