

Short communication

Diminished expression of the costimulatory ligand CD80 (B7.1) gene correlates with antigen-specific cellular unresponsiveness in rhesus monkeys with lymphatic filariasis

Guillermo H. Giambartolomei^{a,1}, Barbara L. Lasater^a, François Villinger^b,
Vida A. Dennis^{a,*}

^a Department of Parasitology, Tulane University Medical Center, Tulane Regional Primate Research Center,
18703 Three Rivers Road, Covington, LA 70433, USA

^b Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA

Received 12 April 2000; received in revised form 23 August 2000; accepted 11 September 2000

Keywords: Rhesus monkey; *Macaca mulatta*; *Brugia malayi*; filariasis; B7.1; B7.2

Lymphatic filariasis, caused by *Wuchereria bancrofti* and *Brugia malayi*, affects an estimated 120 million people in the tropical and subtropical regions of the world. The disease persists as a major cause of morbidity in these regions (Ottesen and Ramachandran, 1995). The clinical manifestations of lymphatic filariasis are very broad, and they are considered, at least in part, a reflection of the type of immune response mounted by the host against the parasite and parasite products (Ottesen, 1992). One of the hallmarks of lymphatic filariasis is antigen-specific cellular unresponsive-

ness that is typically seen in patients that are asymptomatic and microfilaraemic or in those that are circulating antigen positive and microfilaraemic (Dimock et al., 1996). Cells from these patients are also unable to produce IL-2 (Dimock et al., 1996) and IFN- γ (Freedman 1998) as induced by filarial antigens.

Our laboratory has actively characterized a non-human primate model of filariasis namely rhesus monkeys infected with *B. malayi*. Salient findings of these studies showed that chronic infection was established in these animals and, similar to chronic infection in humans, the monkeys exhibit pathological, parasitological and immunological responses (Dennis et al., 1998; Giambartolomei et al., 1998). Two clinically distinct groups can be identified, one being asymptomatic and essentially microfilaraemic. Another group of chronically infected monkeys display lymphedema

* Corresponding author. Tel.: +1-504-8716267; fax: +1-504-8716390.

E-mail address: vida@tpc.tulane.edu (V.A. Dennis).

¹ Present address: Instituto de Estudios de la Inmunidad Humoral, Facultad de Farmacia y Bioquímica, Junín 956, 4 Piso. 1113. Buenos Aires, Argentina.

and the majority of the animals within this group are amicrofilaremic. Nevertheless, we were unable to determine the infection status of the amicrofilaremic or microfilaremic animals using a circulating antigen detection assay (unpublished data). Corresponding to these clinical observations, the first group of monkeys showed a characteristic lack of antigen specific proliferative responses (non-responders, NRM) whereas the second group showed vigorous proliferative response to antigen (responder, RM). Our laboratory further correlated the lack of response with diminished production of IL-2 and IFN- γ and lack of induction of IL-2R⁺ on T cells in NRM (Giambartolomei et al., 1998).

The CD28/B7 receptor/ligand system is one of the most important costimulatory pathways (reviewed by Lenschow et al., 1996). B7, which is present mainly on antigen-presenting cells (APC) is a ligand for CD28 expressed on T cells. The B7 antigen is present in at least two isoforms on APC, B7.1 (CD80) and B7.2 (CD86). Both CD80 and CD86 can provide costimulation to T cells for proliferation and IL-2 production. These ligands play a crucial role in the establishment of antigen-driven immune responses. The significance of costimulatory signals is emphasized by the induction of antigen-specific unresponsiveness or anergy when T cells encounter antigens in the absence of costimulation (Lenschow et al., 1996). It also has been shown that immunoregulatory cytokines may influence the development of the Th1 versus Th2 immune responses by altering the level of expression of costimulatory ligands on APC (Kuchroo et al., 1995).

This study was initiated to assess the role that costimulatory ligands may play in the parasite-specific cellular unresponsiveness in rhesus monkeys with lymphatic filariasis. Using RT-PCR, we measured the relative expression of CD80 and CD86 in PBMC that were stimulated *in vitro* with *B. malayi* antigens (BmA). Nine monkeys chronically infected with *B. malayi* and three uninfected monkeys (UM) were studied. Animal infection, as well as their clinical and parasitological status were reported elsewhere (Giambartolomei et al., 1998). At the initiation of the experiment, five animals had been chronically infected for 288 weeks, and four animals for 192 weeks.

The procedures for PBMC isolation, BmA preparation and blastogenesis have all been previously published (Dennis et al., 1998; Giambartolomei et al., 1998). Briefly, PBMC were isolated on Ficoll-Hypaque gradients and final cell suspensions were made in RPMI-1640 supplemented with 10% heat inactivated human AB serum. For blastogenesis assays, PBMC (2×10^6 per ml) were cultured with either BmA (10 μ g/ml) or Con A (8 μ g/ml). Control media only cultures (unstimulated) were run simultaneously. Results were expressed as stimulation index (counts per min (CPM) of stimulated cultures divided by CPM of unstimulated cultures). Stimulation indices > 2 were considered as a positive specific response.

PBMC (2×10^6 cells) were cultured in round-bottom polypropylene tubes (Sarstedt, Nümbrecht, Germany) in the presence of RPMI 1640 containing 10% heat-inactivated fetal calf serum, BmA (10 μ g/ml) or Con A (8 μ g/ml) in a volume of 1 ml. Cultures were incubated at 37°C in a humidified atmosphere (5% CO₂) for 24 h. At the end of the incubation cells were centrifuged at $400 \times g$ at 4°C and processed immediately for RNA extraction. Each RT-PCR was performed from 250 ng of total RNA as previously described (Giambartolomei et al., 1998). Primer and probe sequences specific for macaque glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have been published elsewhere (Villinger et al., 1995). The primers and probes used for the costimulatory ligands were as follows:

CD80(B7.1), 5'-TATGGGCCACACACGGA-GGCAGG-3' and

5'-TTCAGGATCTTGGGAACTGTTG-3'

5'-TCACCTCTCCTGGTTGGAAAATGG-3' (probe)

CD86(B7.2), 5'-TTCAGATCAAGGACAAG-GGCTT-3' and

5'-GGGAATGAAACAGACAAGCTGA-3'

5'-CATTCCTGTGGGC(T/C)TTTTGTG-3' (probe).

Results (net intensity of the amplified fragments) were expressed relative to (fold increase) the results obtained from cells cultured in the absence of antigen. Increases > 2 were considered positive. All data were analyzed using the non-paired Student's *t*-test. Significance was assessed at the 0.05 level.

Infected monkeys were divided according to the ability of their PBMC to proliferate *in vitro* to BmA. Three of these monkeys were classified as responder (RM) (S.I. > 2) and six as non-responders (NRM) (S.I. < 2). PBMC from UM showed no response to BmA. In contrast, PBMC from all monkeys proliferated in response to the mitogen Con A with no significant difference observed among the three groups of animals (Fig. 1). CD80 mRNA gene expression was significantly induced by BmA restimulation of cells from 3/3 RM [geometric mean fold increase (GMFI) of 33.88 (24–54) over unstimulated cells] (Fig. 2). However, BmA stimulation of PBMC from 3 NRM failed to induce the expression of CD80 mRNA and only marginally induced it in PBMC of the remaining 3 NRM [GMFI of 2.02 (0.7–6.0)]. Overall, CD80 gene expression in cells from RM was 17-fold greater than those of NRM ($P < 0.001$) (Fig. 2). BmA did not induce CD80 gene tran-

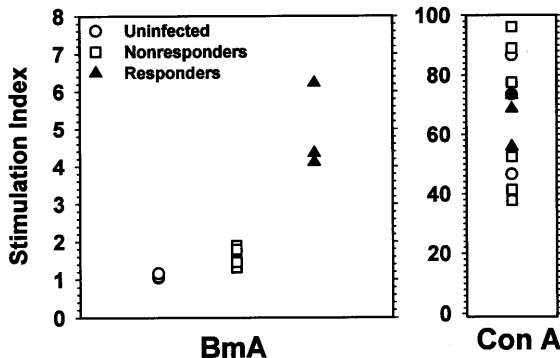


Fig. 1. *In vitro* blastogenesis of PBMC from rhesus monkeys chronically infected with *B. malayi* to BmA (A) and Con A (B). Each data point represents triplicate responses from individual uninfected (open circles), responder (closed triangles) and non-responder (open squares) rhesus monkeys. Results are expressed as stimulation index, which is the CPM of stimulated cultures divided by the CPM of unstimulated cultures. (Data published previously: Giambartolomei et al., 1998).

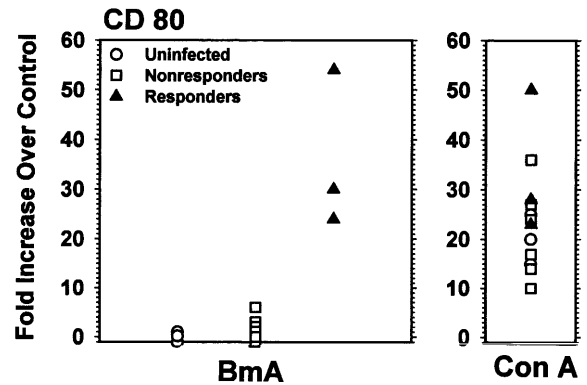


Fig. 2. BmA-induced CD80 (B7.1) mRNA expression in PBMC from rhesus monkeys chronically infected with *B. malayi*. PBMC (2×10^6 per ml) from uninfected (open circles), responder (closed triangles) and non-responder (open squares) rhesus monkeys were stimulated with BmA (10 $\mu\text{g}/\text{ml}$) or Con A (8 $\mu\text{g}/\text{ml}$) for 24 h. The induced mRNA levels of CD80 were determined by RT-PCR. Responses are shown as fold increase over unstimulated PBMC. Each symbol represents the response of PBMC from an individual monkey. All values were normalized with respect to GAPDH mRNA levels.

scription in PBMC from control animals. Con A induced CD80 transcripts in cells from all animals. However, albeit not statistically significant, CD80 expression level in Con A stimulated RM PBMC was higher [GMFI of 28.00 (20–50)] than in NRM PBMC [GMFI of 16.82 (10–36)] and UM PBMC [GMFI of 19.57 (15–25)] (Fig. 2). In contrast, high levels of CD86 mRNA transcripts were constitutively expressed in PBMC from all groups of monkeys irrespective of stimulation or absence thereof (ratios ≤ 1.0). Unfortunately, very limited availability of samples for these retrospective analyses precluded the performance of additional assays to adjust the amount of sample for the quantitative range of the CD86 mRNA determination. A new cohort of monkeys is currently being infected to better address the modulation of costimulatory molecules such as CD80, CD86, CD40, CD154 and CTLA-4 in RM versus NRM monkeys.

Antigen specific responses are initiated via the engagement of the T-cell receptor on the surface of specific T cells with its cognate antigen peptide presented within the context of autologous MHC molecules. Full activation of responder T cells

however requires a secondary signal which is most often delivered via CD28, after its engagement by CD80 or CD86, leading to T cell proliferation and IL-2 production (Lenschow et al., 1996). In addition, the relative density of CD80 and/or CD86 present on the APC is also a determinant of the nature, quality and extent of the immune response generated (Freeman et al., 1995). Yet, CD80 and CD86 expression has been only poorly investigated in antigen specific unresponsiveness during chronic filariasis. Ravichandran et al. (1997) reported no differences in CD80 mRNA levels between microfilaremic patients and individuals with *W. bancrofti* lymphatic obstruction. Baize et al. (1997) also reported similar B7 mRNA levels between microfilaremic and amicrofilaremic patients infected with *Loa Loa*, albeit this study failed to discriminate between CD80 (B7.1) and CD86 (B7.2). Using the rhesus macaque model of lymphatic filariasis, our study clearly demonstrates a strong association between BmA-induced proliferative responses and expression of CD80 but not of CD86. The differences observed between the various studies are unclear at present, but may be partially explained by differences in parasites, clinical status, host species and/or assay setup.

CD86 is considered to be a more effective costimulator of T cells involved in cellular mediated responses (Buelens et al., 1995). The results of the present study, however, indicate high constitutive levels of CD86 expression, even in the absence of antigenic stimulation in PBMC from both RM and NRM. Indeed monocytes/macrophages have been shown to constitutively express CD86 but not CD80 (Azuma et al., 1993). In addition, monkeys may be further prone to relatively elevated constitutive CD86 expression given the finding of a general higher frequency of activated circulating T-cells in PBMC of even healthy non-human primates (Ansari et al., 1994 Bostik et al., 1999). Thus, it is possible that within a background of high levels of CD86 expression, modulation of CD80 expression actually dictates or is associated with efficient T cell response or lack thereof.

CD80 and CD86 expression patterns have been shown to dictate the activation of Th1/Th2 pathways (Kuchroo et al., 1995). These observations

are in line with our previous report about RM PBMC secreting IL-2/IFN- γ in response to antigenic stimulation whereas most NRM PBMC responded mainly with IL-4 and IL-10 (Giambartolomei et al., 1998). However, while IL-10 has been reported to down-regulate the expression of CD80 and CD86 (Buelens et al., 1995), our results did not suggest any correlation between elevated IL-10 production (Giambartolomei et al., 1998) and CD80/CD86 expression in RM and NRM animals. One caveat of the studies reported herein is that the kinetics of expression of the cytokines and costimulatory molecules have not been addressed. Such analyses will however be performed on a new set of monkeys currently being inoculated with *Brugia malayi*. In conclusion, our findings suggest that diminished CD80 gene expression may be another determinant factor in the antigen-specific cellular unresponsiveness of rhesus macaques with chronic lymphatic filariasis.

Acknowledgements

This work was supported by grant RR00164 from the National Center for Research Resources, National Institute of Health. *B. Malayi* adult worms were supplied by NIAID supply contract (AI # 02642), US-Japan Cooperative Medical Science Program. G.H.G is a post-doctoral fellow of CONICET (Argentina).

References

- Ansari, A.A., Mayne, A., Hunt, D., Sundstrom, J.B., Villinger, F., 1994. TH1/TH2 subset analysis. I. establishment of criteria for subset identification in PBMC samples from non-human primates. *J. Med. Primatol.* 23, 102–107.
- Azuma, M., Ito, D., Yagita, H., Okumura, K., Phillips, J.H., Lanier, L.L., Somoza, C., 1993. B70 antigen is a second ligand for CTLA-4 and CD28. *Nature* 366, 76–79.
- Baize, S., Wahl, G., Soboslay, P.T., Egwang, T.G., Georges, A.J., 1997. T helper responsiveness in human *Loa loa* infection; defective specific proliferation and cytokine production by CD4+ T cells from microfilaremic subjects compared with amicrofilaremics. *Clin. Exp. Immunol.* 108, 272–278.

- Bostik, P., Brice, G.T., Mayne, A.E., Villinger, F., Lewis, M.G., Ansari A.A. 1999. Increased proliferation of CD4+ T lymphocytes in different species of SIV infected non-human primates is associated with lack of or delayed disease progression. *J. Immunol.* (submitted).
- Buelens, C., Willems, F., Delvaux, A., Pierard, G., Delville, J.P., Velu, T., Goldman, M., 1995. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells. *Eur. J. Immunol.* 25, 2668–2672.
- Dennis, V.A., Lasater, B.L., Blanchard, J.L., Lowrie, R.C., Jr., Campeau, J.R., 1998. Histopathological, lymphoscintigraphical and immunological changes in the inguinal lymph nodes of rhesus monkeys during the early course of infection with *Brugia malayi*. *Exp. Parasitol.* 89, 143–152.
- Dimock, K.A., Eberhard, M.L., Lammie, P.J., 1996. Th1-like antifilarial immune responses predominate in antigen-negative persons. *Infect. Immun.* 64, 2962–2967.
- Freedman, D.O., 1998. Immune dynamics in the pathogenesis of human lymphatic filariasis. *Parasitol. Today* 14, 229–234.
- Freeman, G.J., Boussiotis, V.A., Anumanthan, A., Bernstein, G.M., Ke, X.Y., Rennert, P.D., Gray, G.S., Gribben, J.G., Nadler, L.M., 1995. B7-1 and B7-2 do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. *Immunity* 2, 523–532.
- Giambartolomei, G.H., Lasater, B.L., Villinger, F., Dennis, V.A., 1998. Diminished production of T helper 1 cytokines and lack of induction of IL-2R+ T cells correlate with T-cell unresponsiveness in rhesus monkeys chronically infected with *Brugia malayi*. *Exp. Parasitol.* 90, 77–85.
- Kuchroo, V.K., Das, M.P., Brown, J.A., Ranger, A.M., Zamvil, S.S., Sobel, R.A., Weiner, H.L., Nabavi, N., Glimcher, L.H., 1995. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 80, 707–718.
- Lenschow, D.J., Walunas, T.L., Bluestone, J.A., 1996. CD28/B7 system of T cell costimulation. *Ann. Rev. Immunol.* 14, 233–258.
- Ottesen, E.A., 1992. Infection and disease in lymphatic filariasis: an immunological perspective. *Parasitology* 104, S71–S79.
- Ottesen, E.A., Ramachandran, C.P., 1995. Lymphatic filariasis infection and disease: Control strategies. *Parasitol. Today* 11, 129–131.
- Ravichandran, M., Mahanty, S., Kumaraswami, V., Nutman, T.B., Jayaraman, K., 1997. Elevated IL-10 mRNA expression and downregulation of Th1-type cytokines in microfilaraemic individuals with *Wuchereria bancrofti* infection. *Parasite Immunol.* 19, 69–77.
- Villinger, F., Brar, S.S., Mayne, A., Chikkala, N., Ansari, A.A., 1995. Comparative sequence analysis of cytokine genes from human and nonhuman primates. *J. Immunol.* 155, 3946–3954.