TD-BF03: Cytokines & Chemokines

Articles 1

From Ahmed Medhat, Magda Shehata, Kim Bucci, Shoari Mohamed, Ahmed Diab Enas Dief, Saad Badary, Hesham Galal, Mohamed Nafeh, and Christopher L. King. *Journal of infectious disease* 1998, 178:512-519.

I-Introduction

Epidemiologic studies of human schistosomiasis suggest that acquired immunity gradually develops with age. What is known is that persons with increased resistance to reinfection after chemotherapeutic cure have elevated serum levels of schistosome adult worm (SWAP) specific IgE, IgA directed to the 28 kDa schistosome antigen glutathione-S-transferase, peripheral blood eosinoophilia. Conversely, increased serum levels of IgG4, IgG2 and IgM directed against adult worms antigens are associated with an increase susceptibility to reinfection. Antigen-specific IgG4 and IgG2 have been postulated to block IgE-mediated killing of the parasite. It is possible that elevated levels of IgE and peripheral blood eosinophilia are markers of anti-parasite cell-mediated immunity.

II-Material and Methods

Patients. A cohort of 145 male children aged 9-15 years were selected on the basis of their willingness to participate. Midday urine was obtained on 2 consecutive days at the beginning of the study and at 3, 6 and 12-18 months later. Any individual found infected with shistosomiasis was treated with 40 mg/kg praziquanted. Between 12 and 18 months after treatment, ± 20 ml peripheral blood was obtained to prepare peripheral blood mononuclear cell (PBMC).

Antigen. SWAP antigen Schistosoma Hematobium was prepared as a saline extract form.

PBMC. All studies were performed on fresh PBMC sparated by density-gradient centrifugation on ficoll-hypaque from venous blood and resustended in RPMI medium. PBMC were cultured at 2 10^6 cells/ml and 50 μg of SWAP/ml at 37°C fro 24-72 hours and supernatants were tested for cytokines production.

Cytokines assay: cytokine levels in cell supernatants were measured by ELISA and expressed in picograms/ml. Tests were done using specific antibodies for capture and detection of different cytokines.

III-Results

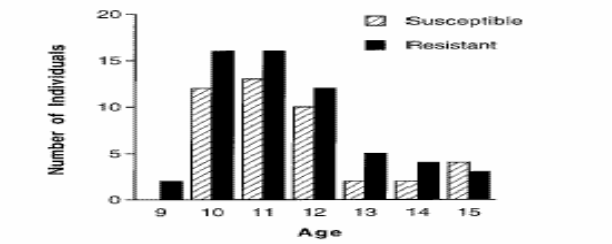


Figure 1. Age distribution of susceptible and putatively resistant subjects in study cohort.

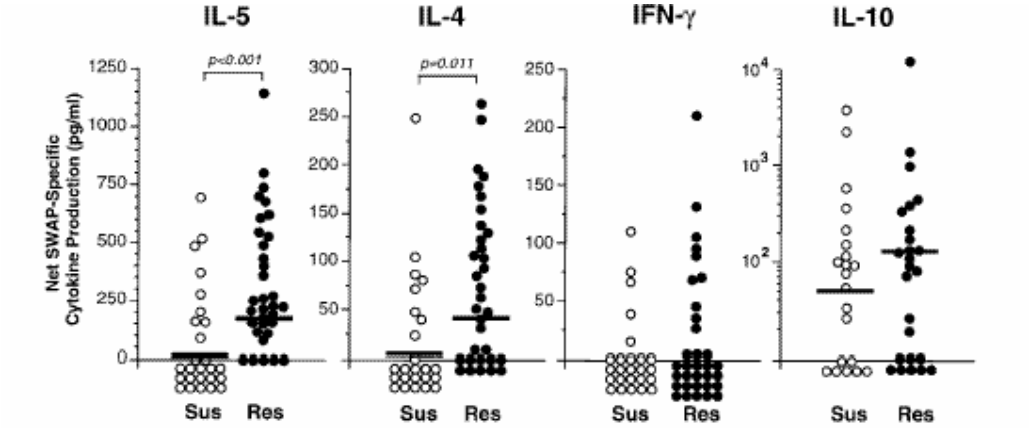


Figure 3. Cytokine production by culture supernatants between susceptible (Sus) and putatively resistant (Res) subjects. Values represent net schistosome adult worm antigen (SWAP)–induced cytokine production in culture supernatants of 2×10^6 peripheral blood mononuclear cells/mL in 0.5-mL cultures harvested 72 h (IL-5, IL-10, and interferon [IFN]-γ) or 24 h (IL-4) after addition of antigen. Each dot represents mean value of single or duplicate cultures from 1 patient. *P* values were calculated by Student's *t* test using log-transformed data.

Question 1

What conclusion can be drawn from these results? What are the cytokines that play a role in sensibility/resistance to *S. hematobium*? Suggest others mechanisms that couls play a role in immunity to *S. hematobium*.

Article 2

From Lin Chen, Fujiro Sendo. *Parasitology International* (50) 2001:139-143.

I-Introduction

The mouse experimental cerebral malaria (ECM) model mimics certain aspects of human cerebral malaria. Susceptible CBA_NSlc mice developed a neurologic syndrome 1 to 2 weeks after PbA infection and most of them died within this period. The major difference between ECM and human cerebral malaria CM is the cells sequestered in the small vessels. In ECM, they are monocytes and in human CM , they are parasitized erythrocytes. Cytokines, including Th1 cytokines IL-2 and IFN-γ and inflamatory cytokines TNF-α and IL-6, especially excessive production of TNF-α, play very important roles in the pathogenesis of this fatal disease. Since neutrophils do not sequester in brain blood vessels, little attention has been paid to them. Recently, neutrophils have been involved in the disease because their depletion using a mAb prevented the early development of ECM and dramatically decreased the sequestration of monocytes and microhemorrhage in the brain.

II-Material and Methods

Mice and infection. CBA_NSlc mice were infected by intraperitoneal injection of 10^6 *Plas modium berghe*i parasitized RBC.

Cells. Peritoneal exudates neutrophils (PEN) and peritoneal exudates macrophages (PEM) were obtained from CBA_NSlc mice after i.p. injection of with 4,5 ml 3% proteose peptone 24 hours before or not injection i.p. with 106 parasitized RBC. Twenty-four hours after infection, PEM and PEN were harvested by peritoneal lavage and adherent cells were obtained after 3 h of incubation at 37°C. The non-adherent cells were removed by exhaustive

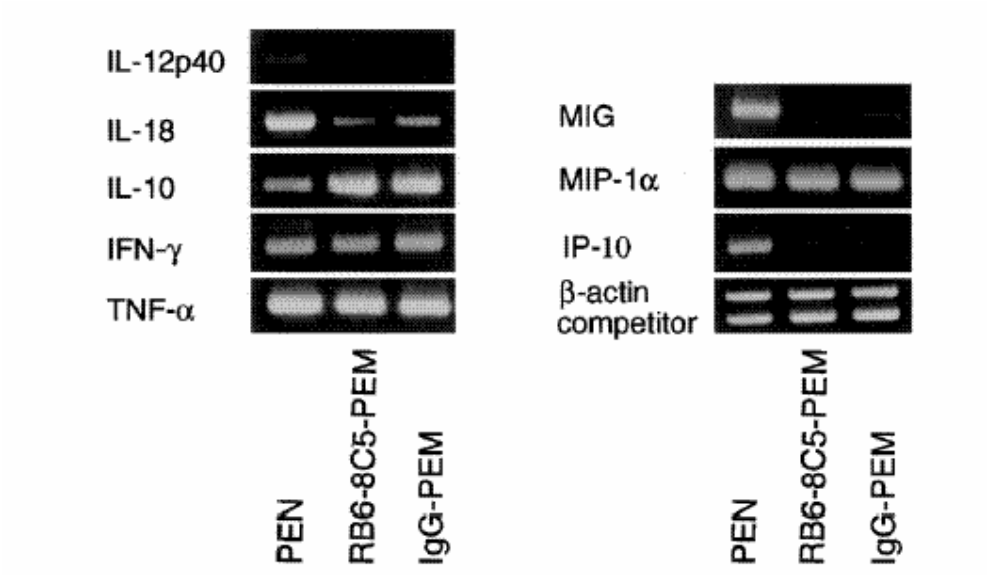
washing. The purities of the PEN and PEM preparations were 95%.

Neutrophil depletion. To depletes in neutrophils mice were treated 24 hours after injection of proteose peptone with i.p. with 0.25 mg RB6-8C5 mAb which selectively depletes neutrophils or with control IgG.

mRNA isolation. Total RNA was extracted immediately from purified PEN and PEM, without antigen stimulation in vitro, using ISOGEN NIPPON GENE.

III-Results

Figure 1. The expression of cytokine and chemokine mRNAs by PEN and PEM.



The total RNA was reverse transcribed by AMV reverse transcriptase XL.The actins of different samples were adjusted to the same levels. PCR amplification was done using primers for actin, IFN-γ, TNF-α, IL-10 and IL-12p40,IL-18, MIG, MIP-1α and IP-10. There were 35 PCR cycles for cytokines and 30 for chemokines. With these numbers of cycles, the amounts of PCR products had not reached saturation. Density was evaluated and expressed as cytokine or chemokine band intensity divided by actin band intensity.

Table 1
The expression of cytokine and chemokine mRNAs by PEN and PEM[a,b]

Cytokines and chemokines	PEN	RB6-8C5-PEM	IgG-PEM
IL-12p40	0.32	ND	ND
IL-18	1.20	0.40*	0.70*
IL-10	0.60	1.20	1.20
IFN-γ	1.00	0.80	1.10
TNF-α	1.70	1.58	1.50
MIG	1.60	0.05**	0.20**
MIP-1α	1.25	1.10	1.05
IP-10	0.80	0.20	0.15

[a] The original data are shown in Fig. 1. Each value indicates target/β-actin density. The result shows a representative of three experiments. ND: not detectable.
[b] '*' and '**' indicate $P < 0.05$.

Question 2

What are the cytokines and chemokines expressed by neutrophils and macrophage after infection? Explain the effect of neutrophil depletion on cytokines expression.

Article 3

From Oscar Bruna-Romero, John Schmieg, Margarita Del Val, Michael Buschle, and Moriya Tsuji

I-Introduction

Cell-mediated immunity plays a crucial role in the control of many infectious diseases, necessitating the need for adjuvants that can augment cellular immune responses elicited by vaccines. It is well established that protection against malaria is dependant on strong $CD8^+$ T cell responses targeting *Plasmodium* spp liver. Given the need for vaccine that can elicit strong T cell responses, much recent research has been focused on the evaluation of various molecules as possible adjuvants capable of enhancing vaccine-elicited T cell responses. These molecules include a number of cytokines, such as IL-12 and GM-CSF, as well as certain microbial products, such as unmethylated CpG DNA, that are known to stimulate and/or support cell mediated immune responses.
Dendritic cell-derived CC chemokine 1 (DC-CK1 also known as MIP-4/PARC/AMAC-/CCL18) is a recently identified human CC chemokine produced by dendritic cells. DC-CK1 induces both intracellular Ca2+ mobilization and chemotaxis in $CD4^+$ and $CD8^+$ naïve T cells but not in T cells bearing the activation marker CD45RO and not in nonlymphocytic cells such as monocytes and granulocytes. DCCK1 is also chemotactic for naive B cells. Given the activity of DC-CK1 on naive lymphocytes and its production by dendritic cells, it is possible that this chemokine is involved in the induction of primary T and B cell responses. In vivo role for DC-CK1 in the establishment of primary T cell responses could be usefull to the use of this chemokine as an adjuvant for vaccines against malaria as well as other diseases in which cellular immune responses are important.

II-Material and Methods

Animals and parasites. Six- to 8-wk-old female BALB/c mice were used for most experiments. IL-12p40- deficient mice and CD40-deficient mice of BALB/c background were

purchased from The Jackson Laboratory (Bar Harbor, ME). IFN-γ receptor deficient mice with an H-2d background were generated. *P. yoelii* (17XNL strain) was maintained by alternate cyclic passages in *Anopheles stephensi* mosquitoes and Swiss-Webster. Sporozoites obtained from dissected salivary glands of infected mosquitoes 2 wk after their infective blood meal were used for immunization as well as challenge of the mice.

Construction and screening of recombinant adenoviruses. Recombinant adenovirus expressing the *P. yoelii* circumsporozoite (CS) protein (AdPyCS) was constructed by first inserting a PCR fragment containing the open reading frame for aa 1–356 of the CS protein into a CMV expression cassette containing the CMV immediate early promoter, followed by transfer of this cassette into the adenoviral shuttle vector pMV60. (25). Replication-deficient, human type 5 adenoviruses were generated in 293 cells by homologous recombination of the constructed shuttle plasmid and plasmid pJM17 containing the complete Ad5 _E1_E3 genome.

Immunizations and challenge. In all experiments groups of three to five mice were immunized s.c. with suboptimal doses of immunogen ($2x10^4$ irradiated sporozoites/mouse, $2x10^7$ PFU AdPyCS/mouse, or $5x10^6$ PFU Adpp89-CD8/mouse) with or without doses or recombinant human DC-CK1 protein in a final volume of 100 μl/mouse. Challenge of mice to determine the inhibition of liver stage development was performed by i.v. injection of 10,000 viable sporozoites into the tail vein 2 wk after immunization. The outcome of the challenge was determined 42–44 h later by measuring the parasite burden in the liver of the mice using a quantitative real-time RT-PCR method.

Detection and quantification of P. yoelii rRNA sequences by RT and real-time PCR. Quantification of *Plasmodium* 18S rRNA sequences was performed using a recently developed real-time RT-PCR technique. Briefly, total RNA (2 μg) from the livers of mice challenged with 10,000 viable sporozoites 42–44 h earlier was reverse transcribed, and an aliquot of the resulting cDNA (133 ng) was used for real-time RT-PCR amplification of *P. yoelii* 18S rRNA sequences.

Quantification of epitope-specific CD8_ T cells by ELISPOT Assay. The relative number of CS-specific, IFN-γ-secreting CD8+ T cells in the spleens of mice receiving different immunization regimens was determined by direct ex vivo ELISPOT assays. For these assays, we used MHC-compatible A20.2J target cells coated with the Csderived H-2Kd-restricted epitope SYVPSAEQI, which is recognized by CS-specific CD8_ T cells. For quantification of IFN-γ-secreting CD8+ T cells specific for the CTL epitope of the MCMV immediate early protein 1, we used A20.2J target cells coated with the H-2Ld-restricted epitope YPHFMPTNL.

III-Results

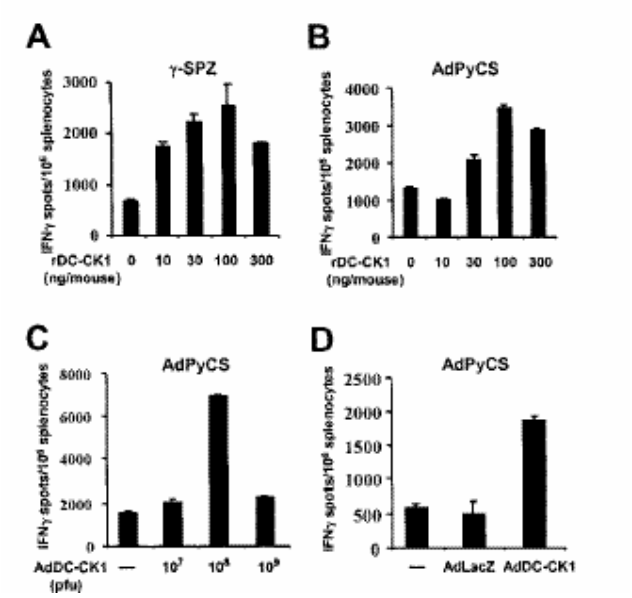
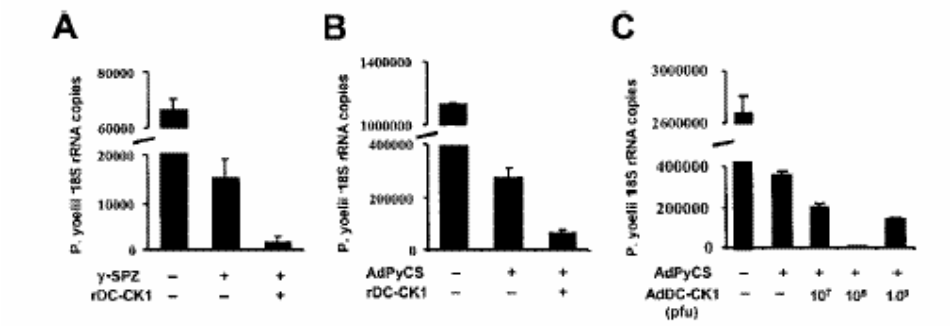


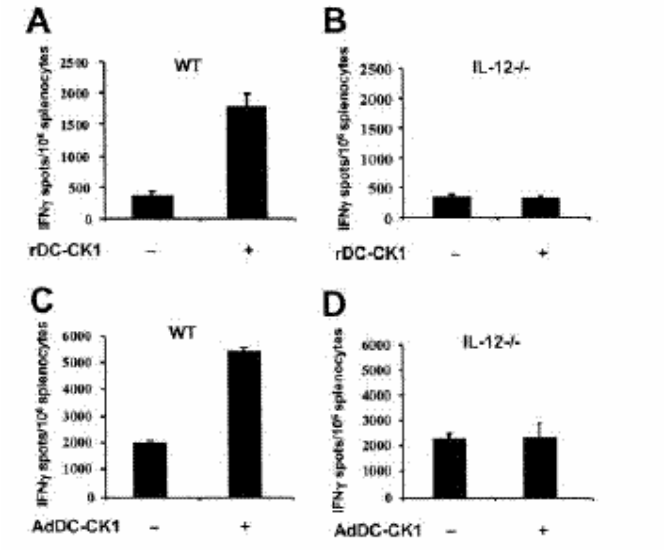
Figure 1. Groups of three BALB/c mice were immunized s.c. with 2 x 10^4 γ-spz (*A*) or 2x 10^7 PFU AdPyCS (*B*) with or without different doses of rDC-CK1 by the same route, 2x10^7 PFU AdPyCS with or without different doses of AdDC-CK1 by the same route (*C*), or 2x 10^7 PFU AdPyCS with or without 10^8 PFU AdDC-CK1 or 10^8 PFU of a control adenovirus, AdLacZ, by the same route (*D*). Two weeks after immunization, splenic lymphocytes were isolated from all mice, and the relative numbers of IFN-γ-secreting, CS-specific CD8+ T cells were determined by ELISPOT assay. The results are expressed as the average ± SD of triplicate cultures. In all figures the data represent one of two or more experiments with similar results.

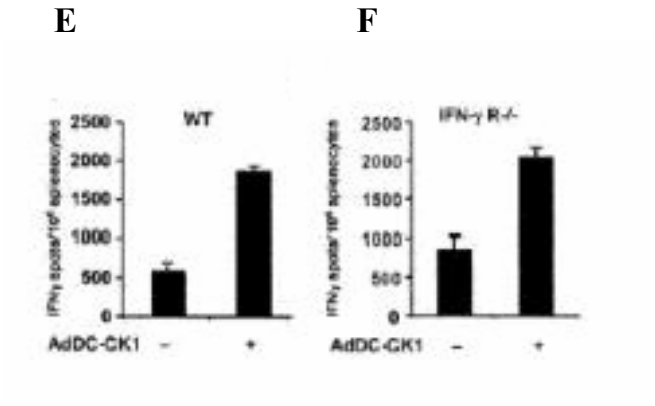
Figure 2



Groups of five BALB/c mice were immunized s.c. with 2 x10^4 γ-spz (*A*) or 2x10^7 PFU AdPyCS (*B*) with or without 100 ng rDC-CK1 or with 2 x10^7 PFU AdPyCS with or without different doses of AdDC-CK1 (*C*). Two weeks after immunization, all mice along with unimmunized controls were challenged i.v. with 10,000 live sporozoites, and the amounts of parasite-specific 18S rRNA in the livers were determined by real time RT-PCR assay. Results are expressed as the average± SD of five mice.

Figure 3





Groups of three WT control mice (*A*) or IL-12-knockout mice (*B*) were immunized s.c. with 2 x 10^7 PFU AdPyCS with or without 100 ng rDC-CK1. Two weeks later, the relative numbers of IFN-γ-secreting, CS-specific CD8+ T cells were determined by ELISPOT assay. Groups of three WT control mice (*C and E*), IL-12-knockout mice (*D*) or IFN-γ Receptor knockout mice were immunized with 2 X 10^7 PFU AdPyCS with or without 10^8 PFU AdDC-CK1 by the same route, knoand the relative numbers of IFN- γ -secreting, CS-specific CD8+ T cells were determined 2 wk later by ELISPOT. Results are expressed as the average ± SD of triplicate cultures.

Question 3

a) What are the effects of administration of DC-K1 on Ag specific CD8 T cell response in immunized mice?
b) Do they protect mice from infection? What experiments allow to answer this question?
c) What cytokines are involved in the protective immune response? Please resume data obtained by doing a schema summarizing the role of DC-K1 in malaria protective responses induced by immunisation with sporozoites.