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Research Note

Human Cord Blood Lymphocytes Reactive to *Schistosoma japonicum* Soluble Egg Antigen

KIMIKO IWAKI[1)], SHIGEKO NAKASHIMA[1)], JUN FU[1)], MARIKO HATO[1)], MAKOTO ITOH[1)], KAORU SUZUMORI[2)], MAKOTO OWHASHI[3)], MASARU MINAI[4)] AND NOBUO OHTA[1)]

[1)]*Department of Medical Zoology, and* [2)]*Department of Obstetorics and Gynecology, Nagoya City University Medical School, 1 Kawasumi, Mizuhocho, Mizuhoku, Nagoya 467, Japan.*
[3)]*Faculty of Integrated Arts and Science, University of Tokushima, 1-1, Minamijosanjimacho, Tokushima 770, Japan.*
[4)]*Yamanashi Institute for Public Health, Fujimi, Kofu 400, Japan.*
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One of the most important phenomena in immune responses of vertebrates is it's fine specificity. Effective immune responses are induced only for antigens with which prior sensitization occurred. In cases of parasitic protozoan infection, however, reports have been accumulated that hosts without any episode of previous infection were, if not all, able to respond to parasite antigens probably by the mechanisms of cross-reaction or *in vitro* priming (Rzepczyk *et al.*, 1989; Tanaka *et al.*, 1993). These phenomena might have been interpreted to be important in host-parasite interactions through enhancing or disturbing protective immunity in hosts.

Immune response is one of the key phenomena during schistosomiasis because the host immunity is tightly involved in both protection and pathogenesis (James and Sher, 1990). Like as many other parasitic helminths, schistosomes seem to have dexterous mechanisms to disturb or escape from host immunity (Butterworth *et al.*, 1982; Sher and Coffman, 1992). Recent observation that the *Schistosoma japonicum* antigens could activate naive B cells in mice suggests presence of a system somehow inducing promiscuous host immune responses (Yamashita *et al.*, 1993). In this study, therefore, we tested whether *S. japonicum* antigens have any stimulatory effects on naive human T cells.

Several samples of human cord blood mononuclear cells (CBMN) obtained at Nagoya City University Hospital were examined. Cells were separated from the whole blood by Ficoll gradient solution method (specific gravity = 1.077). CBMN were stimulated in vitro with purified protein derivative (PPD) (Nihon BCG Seizo, Tokyo) as well as various parasite antigens; soluble egg antigen (SEA) of *S. japonicum* (Ishii and Owhashi, 1982), crude extracts of *Acanthamoeba polyphaga* (A.poly) (Tanaka *et al.*, 1994), *Dermatophagoides pteronyssinus* (Dp) (Kimura *et al.*, 1990) and MSP1 of *Plasmodium falciparum* (Tanabe *et al.*, 1987). PHA-P (Difco, Detroit, USA) and pooled allogeneic cells treated with mitomycin C (Kyowa Hakko, Tokyo, Japan) were used as positive control stimulants. Results of *in vitro* proliferative responses are shown in Fig. 1. As was expected, only faint responses were observed for the stimulation by PPD, A.poly, Dp and MSP1, while strong responses were observed for positive control stimulants, PHA-P and mixed lymphocyte culture reaction. Unexpectedly, 11 of 12 CBMN tested (91.7%) showed positive prolif-

Correspondence: Nobuo Ohta
岩城喜美子[1]，中島成子[1]，付　軍[1]，羽藤真理子[1]，伊藤　誠[1]，鈴森　薫[2]，大橋　真[3]，薬袋　勝[4]，太田伸生[1]（[1]名古屋市立大学医学部医動物学教室，[2]同産婦人科学教室，[3]徳島大学総合科学部，[4]山梨県衛生公害研究所
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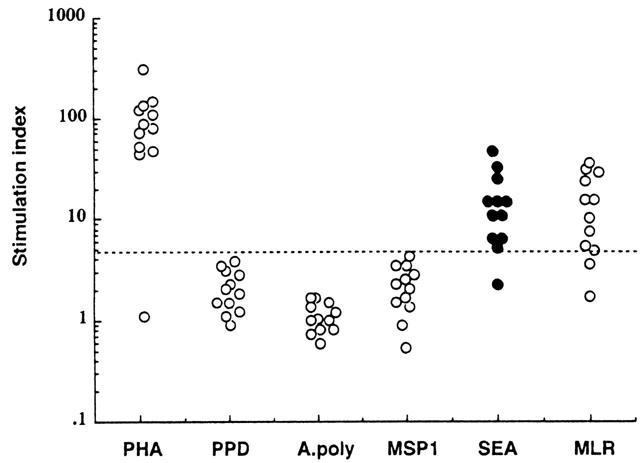


Fig. 1 Proliferative responses of cord blood mononuclear cells to a mitogen, alloantigens and parasite antigens *in vitro*. Twelve cord blood cells were tested, and results are shown in stimulation index calculated as follows; count per minute of test culture/count per minute of negative control culture. A tentative cutoff for positive response is shown in a dotted line. PHA, phytohemagglutinin; PPD, purified protein derivative; A.poly, *Acanthamoeba polyphaga*; MSP1, MSP1 of *Plasmodium falciparum*; SEA, soluble egg antigen of *Schistosoma japonicum*; MLR, mixed lymphocyte reaction stimulated by pooled allogeneic cells.

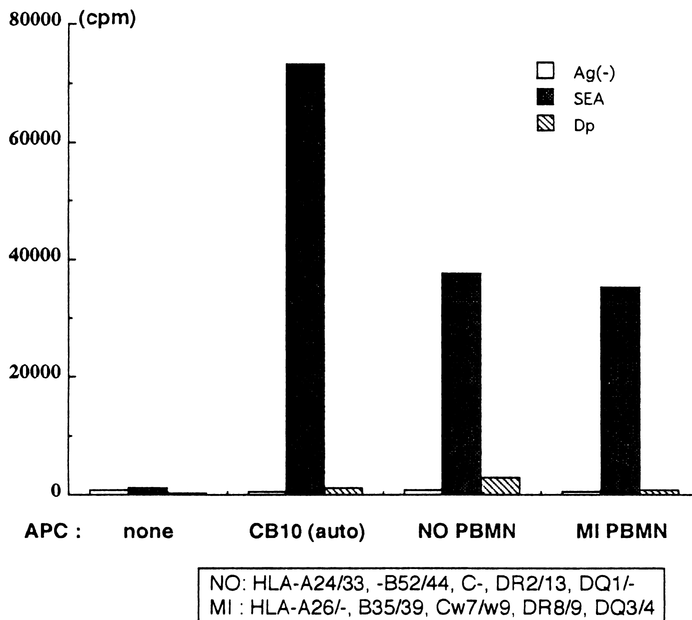
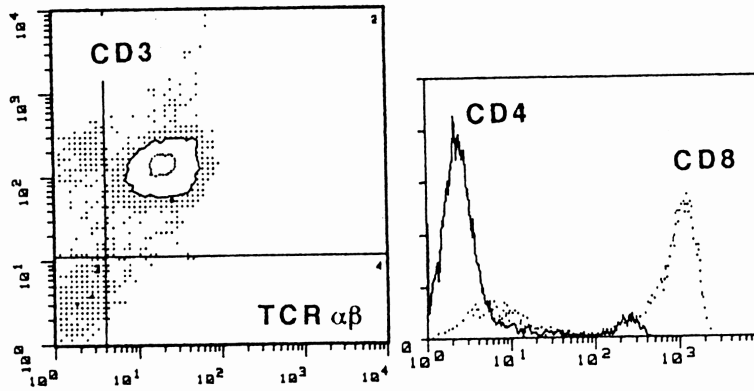
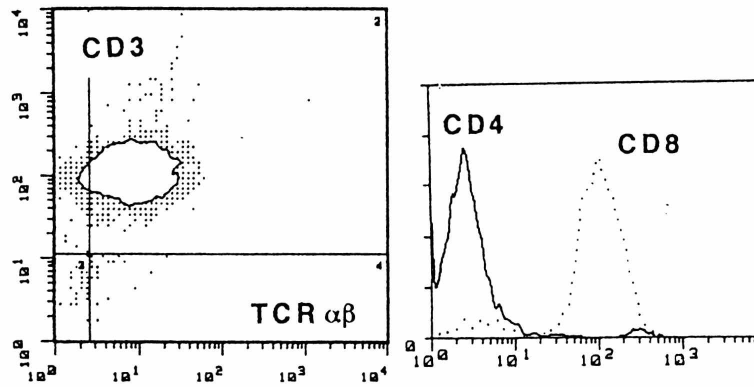


Fig. 2 Antigen presenting cell-dependent proliferative responses of an SEA-reactive T cell line isolated from cord blood mononuclear cells. A fine specificity was observed for SEA, however, vigorous responses were observed not only in the presence of autologous APC, but also in the presence of allogeneic APC of which HLA specificities were completely non-identical.

A. CB10 anti-SEA



B. CB11 anti-SEA



C. CB14 anti-SEA

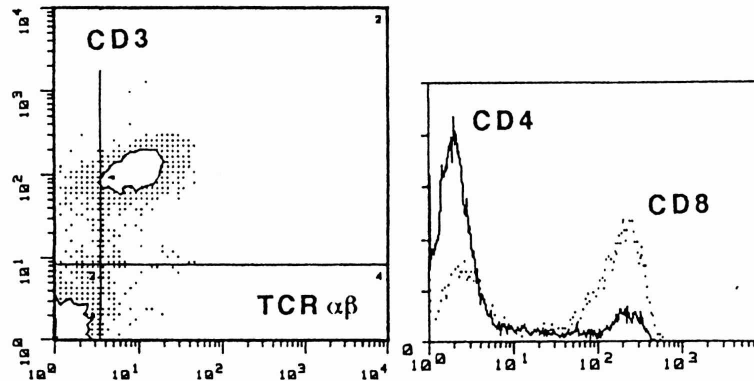


Fig. 3 Surface phenotypes of SEA-reactive blastoid cells derived from cord blood. All three cells tested were $CD3^+$, $CD4^-$, $CD8^+$ and TCR $\alpha\beta^+$. The phenotypes were reconfirmed by repeated testing.

eration to SEA in which a tentative cutoff was set at stimulation index of 5.0. We tested two different lots of SEA which were prepared independently, and similar strength of proliferative responses were observed for both two antigen samples (data not shown).

The presence of SEA-reactive cells in cord blood was confirmed by establishing SEA-reactive blastoid cells *in vitro* by the method described elsewhere (Ohta *et al.*, 1988). We generated blastoid cells from three CBMN. The specificity of those blastoid cells to SEA was checked, and representative results are shown in Fig. 2. SEA-driven proliferation was dependent on the antigen-presenting cells (APC). However, the details in the restricting elements are not fully elucidated because even APC from HLA-nonidentical donors did cooperate enoughly with blastoid cells and resulting vigorous proliferation to SEA (Fig. 2).

It is unlikely that SEA contained mitogenic or super-antigenic activities according to following results. Firstly, time-course kinetics of SEA-driven proliferation was maximum on day 7 (data not shown), and this was similar to that of conventional antigen rather than that of mitogen in which the peak-response was observed on day 3. Second, surface phenotypes of the blastoid cells were almost homologous; $CD3^+$, $CD4^-$, $CD8^+$ and TCR $\alpha\beta^+$ (Fig. 3). Such shifted phenotypes are against a possibility that SEA has super-antigen activities. The phenotypes observed were unusual because the soluble antigen-reactive cells were $CD8^+$ but not $CD4^+$. Considering the unique surface phenotypes, those T cells might have been induced by an unknown mechanism distinct from cross-reaction or *in vitro* priming.

Precise functions of the T cells are not known. Recent reports have suggested that a subtype of $CD8^+$ T cells carry helper function in their cytokine production profiles (Maggi *et al.*, 1994). In the present study, the SEA-reactive $CD8^+$ T cells produced detectable level of interleukin 4 (IL-4) and interferone gamma (IFN-γ) (data not shown) suggesting their helper activity. Although IL-4 production was observed in the $CD8^+$ dominant T cells, a small amount of $CD4^+$ T cells did exist. It is, therefore, still not conclusive whether SEA-reactive $CD8^+$ cells produced the cytokine of helper activity, and further examination using cloned T cells is required. Such T cells are supposed to be in a lineage of double negative helper T cells functioning in a situation where contribution of $CD4^+$ helper T cells is not enough (Erard *et al.*, 1993). SEA-reactive $CD8^+$ T cells may have somehow protective roles in immunologically naive hosts during schistosome infection through their helper functions. Since SEA-reactive cells observed were $CD8^+$, we assumed that those T cells were HLA class-I-restricted as was observed in mice (Le Gros *et al.*, 1994). Our results that the SEA-specific $CD8^+$ T cells were APC-dependent suggest a tight involvement of HLA molecules in their responses. It is still not conclusive about their situation of HLA-allorestriction because only a limited number of allogeneic APC were tested. It is important to analyze how those T cells were induced *in utero*, and why such T cell repertoire have to be prepared. Analysis of restricting elements and detecting recognition molecules are underway.

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