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Identification of a Novel T-Cell Epitope in Soluble Egg Antigen of *Schistosoma japonicum*

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Identification of T-cell epitopes harbored in soluble egg antigen (SEA) of *Schistosoma japonicum* and study of the immunological properties are essential for understanding the immunopathology and the control of schistosomiasis. As a follow-up to our previous work, the 66- to 80-kDa fragment from SEA was partially digested with protease, fractionated by reverse-phase high-pressure liquid chromatography, and found to be carrying a peptide which stimulated proliferation and gamma interferon (IFN-γ) production of Th1 clones specific to SEA. Sequence analysis showed that the peptide was composed of 12 amino acids lined up as DLAVELAYLGNL. A synthetic homologue induced proliferation and IFN-γ and interleukin-2 (IL-2) production, but not IL-4 or IL-6 production, by the Th1 clones as well as by the spleen cells from SEA-immunized mice, thus indicating that the peptide carries a Th1 epitope of SEA.

In schistosomiasis, granulomatous inflammation surrounding the parasite eggs is preceded by a hypersensitivity reaction of $CD4^+$ T helper (Th) cells specific to schistosome soluble egg antigen (SEA). The host's immunity against schistosome infection is also mainly mediated by the specific $CD4^+$ Th cells (4, 9). Activation of the $CD4^+$ Th cell is dependent on recognition of the SEA-derived peptides (epitopes) which are bound to major histocompatibility complex class II molecules and presented by antigen-presenting cells (APCs) (7). Consequently, identification of these T-cell epitopes represents a pivotal step for the study of pathogenesis and immunity and especially for the development of vaccine against schistosomiasis. For *Schistosoma japonicum*, many vaccine strategies have focused on defense against invasion of cercariae or reducing the burden of worms. But we and other colleagues have shown that the pathogenesis of schistosomal infection is mainly caused by a hypersensitivity response of the host to the antigen of the parasite's eggs, resulting in hepatic and intestinal granuloma formation around deposited eggs and subsequent fibrosis (13, 26). Immunization using SEA from *Schistosoma mansoni* has been shown to provide immunity in mice, thus protecting the mice from challenge by *S. mansoni* cercariae. This protective immunity was characterized as a SEA-specific T-cell proliferation accompanied by gamma interferon (IFN-γ) and interleukin-2 (IL-2) production and cytotoxic $CD8^+$ T-cell activation, which contributed to a marked reduction in the number of granulomas and the amount of fibrosis, leading to survival of the mice (2, 17).

T-cell-specific epitopes of egg antigen in *S. mansoni* have been extensively studied, but identification of the epitope in the egg antigen of *S. japonicum*, compared with identification of that found in the worm antigen, remains inadequate. Our laboratory has established Th1 clones ($CD3^+$, $CD4^+$, and $CD8^-$, T-cell receptor-αβ$^+$ [TCRαβ$^+$], TCRγδ$^-$, Vβb10$^+$) from SEA-immunized C3H/He mice as probes to identify novel epitopes of the egg antigen (27). These clones are specific to a range of SEA components from 51 to 80 kDa. Taking this approach, we showed that F6, a fragment (66 to 80 kDa) isolated from SEA of *S. japonicum* by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, induced proliferation of the B1, B21, and A25 Th1 clones (27). The proliferation was F6 specific and syngeneic APC dependent, which suggested that F6 harbored the Th1 epitope. To identify the Th1 epitope, F6 was subjected to partial digestion with *Achromobacter* protease I (WAKO, Osaka, Japan), which cleaves peptides at the carboxyl end of Lys residues. The digested fragments were then fractionated by reverse-phase high-pressure liquid chromatography (Waters, Milford, Mass.) with C_{18} column 218TP54 and eluted with a gradient of 0 to 56% acetonitrile. A fragment from each peak was tested individually for activity to stimulate proliferation and IFN-γ production of the B1 clone. Among those which showed stimulatory activity, a fragment from peak 15, termed the F6-s15 fragment, was the most potent (Fig. 1). Sequence analysis by pulsed-liquid Edman degradation using a model 473 protein sequencer (Applied Biosystems, Foster, Calif.) showed that the F6-s15 fragment was composed of 12 amino acids lined up as DLARELAYLGNL (registered in the Japanese International Protein Sequence Database under accession no. PC 7100 JIPID), with an aspartic acid at the N terminus and a leucine at the C terminus. A FASTA search with the sequence revealed homology to the AE003460–40 CG13552 gene product (72.73% identity), a protein with 122 amino acids in *Drosophila melanogaster*, as well as to T16118 hypothetical protein F20D6.9 (66.67% identity), a protein with 118 amino acids in *Caenorhabditis elegans*. To study the immunological properties of the F6-s15 fragment, a homologous peptide was synthesized. Meanwhile, a peptide with the same length as F6-s15 and a
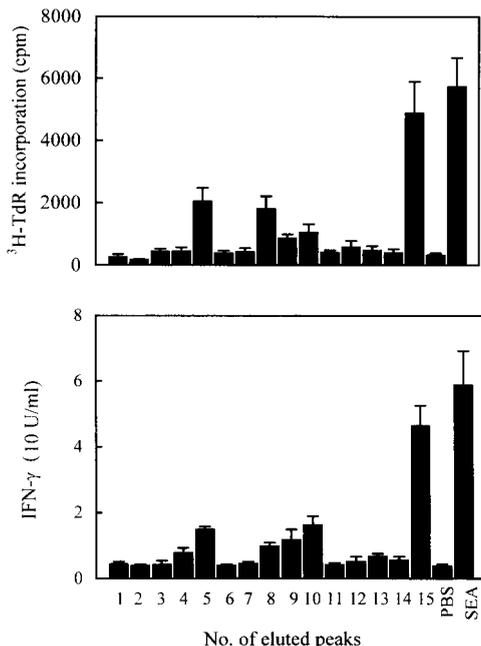
FIG. 1. Response of the B1 clone to fragments fractionated by reverse-phase high-pressure liquid chromatography. The B1 clone was incubated with each fraction (10 μg/ml) individually in the presence of APCs. Proliferation of the B1 clone was expressed in terms of [^{3}H]thymidine (^{3}H-TdR) incorporation after 72 h, which was measured with a liquid scintillation spectrograph. Supernatant of the cultures was collected after 48 h, and IFN-γ was measured. Phosphate-buffered saline (PBS) and SEA (10 μg/ml) were used as negative and positive controls, respectively. Results were expressed as means ± standard deviations of triplicate wells from three experiments performed separately.

sequence randomly selected from bovine serum albumin (BSA26–37; DDSPDLPKLKPD) were synthesized and used as controls to rule out the possibility of antigenic contamination from the synthesis and nonspecific response of lymphocytes.

F6-s15 prompted proliferation of the B1 and B21 clones but not the A25 clone in the presence of 3,000-rad-irradiated syngeneic spleen cells as APCs, whereas SEA induced prolifera-

tion of all the B1, B21, and A25 clones (Fig. 2). Supernatants were collected from the culture of the B1, B21, and A25 clones for IFN-γ, IL-2, IL-4, and IL-6 assay using corresponding cytokine-sensitive cell line WEHI 279 and cytokine-dependent cell lines CTLL-2, CT4S, and 7TD1, respectively. F6-s15 prompted IFN-γ and IL-2 production of the B1 and B21 clones only, whereas SEA prompted cytokine production by all three T-cell clones (Fig. 3a). Cytokine production was also examined at the transcriptional level by reverse transcription-PCR. Specific bands of IFN-γ and IL-2 mRNA were detected in the B1 and B21 clones, but not in the A25 clone, following the stimulation by F6-s15. Upon stimulation by SEA, specific bands of IFN-γ and IL-2 mRNA were detected in the B1, B21, and A25 clones (Fig. 3b). Neither IL-4 nor IL-6 was detected in all three clones at the transcriptional and posttranscriptional levels (data not shown). C3H/He mice (female, 8 weeks old) were immunized with 60 μg of *S. japonicum* SEA/mouse emulsified in complete Freund's adjuvant CFA). After 10 days, the primed spleen cells were prepared for the experiment. Results showed that the primed spleen cells proliferated in vitro following the stimulation by F6-s15 and SEA (Fig. 4a), while F6-s15 induced substantial production of IFN-γ and IL-2 but low levels of IL-4 and IL-6 and SEA induced the production of IFN-γ, IL-2, IL-4, and IL-6 (Fig. 4b). Neither SEA- nor F6-s15-induced specific proliferation or cytokine production was observed in spleen cells from mice immunized with CFA alone (data not shown). The proliferation study showed that the response of the Th1 clones to F6-s15 was analogous to the response to F6, which induced proliferation of the B1 and B21 clones but not the A25 clone (27), indicating that the F6-s15 peptide represents an epitope of F6. The profile of the cytokine production of the Th1 clones and spleen cells demonstrates that F6-s15 is Th1 specific.

The epitope-induced Th1 response plays an important role in antischistosomal immunity by producing cytokines such as IFN-γ and IL-2. It has been shown that, at an early stage of the infection, the host's response against the parasite is of the Th1 type initially (12, 16). Peripheral lymphocytes from chronic schistosome infections have low proliferative potential and poor IFN-γ production in response to egg antigen (24, 28).
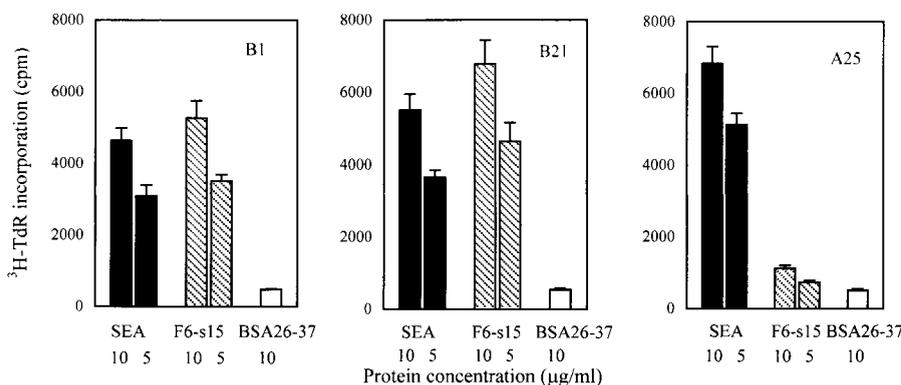

FIG. 2. Proliferation of the B1, B21, and A25 clones of the synthetic F6-s15 peptide. T-cell clones were incubated individually with SEA, F6-s15, and BSA26–37 for 72 h at the concentrations indicated in the presence of APCs. Proliferation of cells was expressed in terms of [^{3}H]thymidine (^{3}H-TdR) incorporation, and background radioactivity from cultures without any antigenic stimulation was subtracted. Results were expressed as means ± standard deviations of triplicate wells from three experiments performed separately.
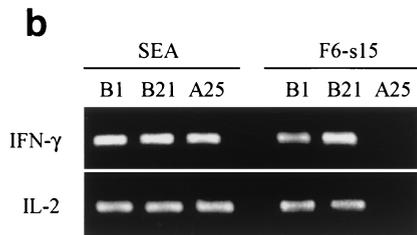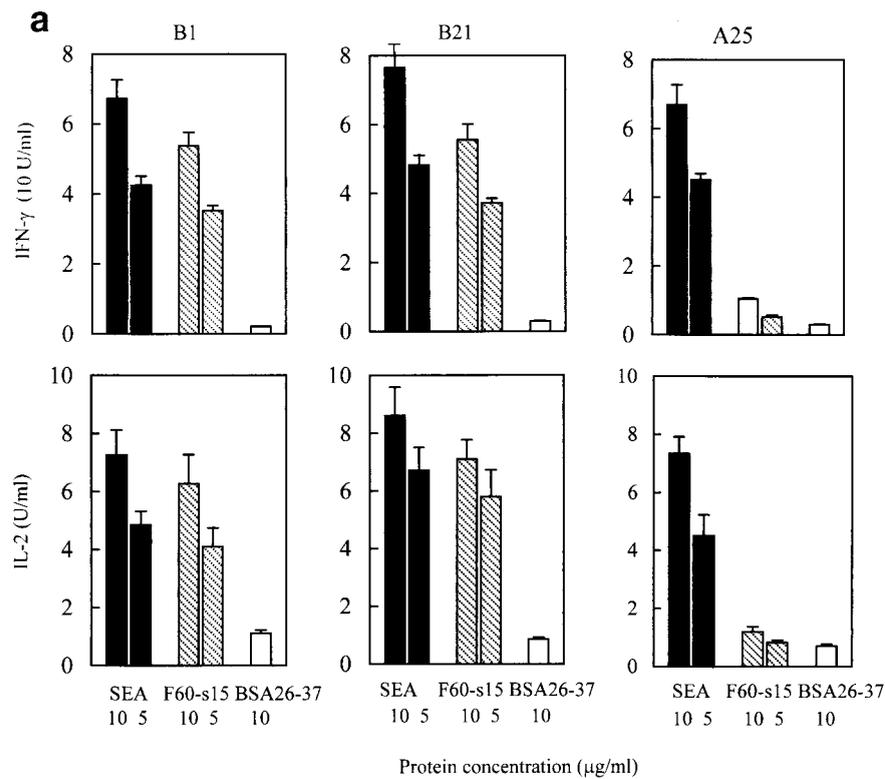
FIG. 3. Cytokine production of the B1, B21, and A25 T-cell clones following stimulation of the synthetic F6-s15 peptide. T-cell clones were incubated individually with SEA, F6-s15, and BSA26–37 for 48 h at the concentrations indicated in the presence of APCs. (a) IFN-γ and IL-2 in the culture supernatants were measured. Results were expressed as means ± standard deviations of triplicate wells from three experiments performed separately. (b) Expression of the cytokine mRNA was detected by reverse transcription-PCR with the corresponding primers. Specific bands were visualized by ethidium bromide staining. Results are representative of three experiments.

Individuals with a high level of IFN-γ were shown to be partially or completely resistant to schistosome infection (20). In addition, IFN-γ has been observed to suppress granuloma formation in both the in vitro and in vivo pulmonary egg infection models (11, 25), as well as to down-regulate the sizes of pulmonary granulomas and the extent of hepatic fibrosis (5, 13). IL-2 is another essential component of antischistosomal immunity. The protective response to *S. mansoni* in mice has been shown to be dependent on CD4^{+} IL-2R^{+} lymphocytes (19). But a decreased IL-2 level in stimulated lymphocytes was found in human schistosomiasis (22, 28) and murine granulomas (21). In addition to that down-regulation, products of *S. mansoni* were shown to interfere with the utilization of IL-2 (14). A low level of IL-2 with consequent IL-2R desaturation is likely to be one of the important mechanisms by which the granuloma T lymphocytes undergo apoptosis (21).

Th1 and Th2 responses are counterregulated (15). Recently, the reduction in granuloma size was observed to be accompanied by a shift from a Th2-type response to a Th1-dominant reaction (3). Following F6-s15 stimulation, the spleen cells from SEA-immunized C3H/He mice mounted a dominant Th1-type response, which was characterized by enhanced

IFN-γ and IL-2 production but not IL-4 or IL-6 production. Following stimulation by SEA, in contrast, the spleen cells mounted a response of mixed Th1 and Th2 type, which was characterized by enhanced production of IFN-γ, IL-2, IL-4, and IL-6. Furthermore, F6-s15 induced a higher level of IFN-γ and IL-2 than did SEA. The different responses of spleen cells to native antigen and to the manipulated one were also observed for *S. mansoni* (1). The variation might be due to the complexity of the SEA, which carries a variety of epitopes both Th1 and Th2 specific. It has been reported that the Th2 cell inhibited Th1 cytokine secretion (6, 18) and that IL-6 directly or indirectly down-regulated IFN-γ production to support the Th2 response (10). Thus, F6-s15 carrying a Th1 epitope induced a higher level of IFN-γ and IL-2 in the spleen cells from SEA-immunized C3H/He mice than did SEA.

But the immune response against schistosome infection is a complicated reaction that involves the recruitment of various epitopes and the activation of different types of cells with different levels of cytokine production. In some reports, Th1 and Th2 were both involved in antischistosomal immunity (8). In other reports, Th1 directly participated in granuloma formation while it provided protection against schistosomes (23).
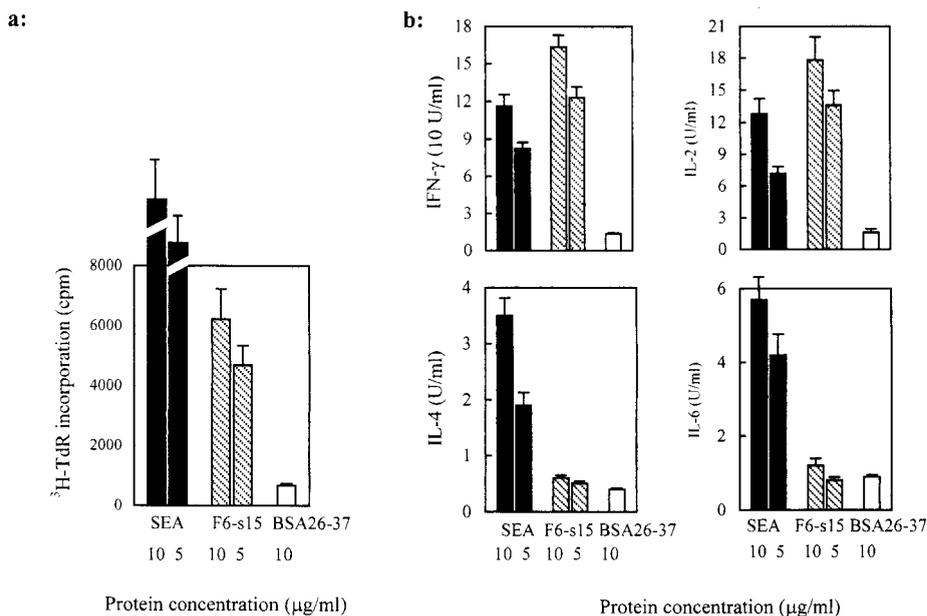
FIG. 4. Response of the spleen cells from SEA-immunized mice to the synthetic F6-s15 peptide. Spleen cells primed with SEA in vivo were incubated individually in vitro with native SEA, F6-s15, and BSA26–37 at the concentrations indicated. (a) Proliferation of cells was expressed in terms of [³H]thymidine (³H-TdR) incorporation, and background radioactivity from a culture without any antigenic stimulation was subtracted. (b) IFN-γ, IL-2, IL-4, and IL-6 in the culture supernatant of the cultures were measured. Results were expressed as means ± standard deviations of triplicate wells from five or six mice for each group.

The observation that Th1 participated in granuloma formation might be due to a hypersensitivity induced by SEA or a response that was not adequately modulated. An effective vaccine candidate should produce an induced immune response of optimal intensity, since the granulomatous inflammation and fibrosis in schistosomiasis are preceded by a hypersensitivity reaction. A balanced response can be achieved by modifying the epitope to form a partial agonist or by seeking a determinant with a mild antigenicity which induces minimum fibrosis to sequester eggs but not to induce severe fibrosis leading to hepatic cirrhosis. In our previous studies, not only the F6 but also the F5 and F7 fragments were found to potently induce proliferation of different T-cell clones. Study of these epitopes will aid in the understanding of the complicated immune response against schistosome infection as well as the design of an effective vaccine.

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