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Separation of IL-4 Production from Th Cell Proliferation by an Altered T Cell Receptor Ligand

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In the presence of antigen presenting cells, a murine T helper (Th) cell specific for murine hemoglobin (Hb) responded to its immunogenic peptide by both cytokine (interleukin-4) secretion and proliferation. An altered Hb peptide with a single amino acid substitution induced only cytokine secretion and did not induce proliferation. Interleukin-1 costimulated and restored the Th proliferative response to normal levels. The altered peptide also supported cognate T cell–B cell interactions indicative of T cell helper function. Thus, this result suggests that the T cell receptor has the capacity of differential signaling.

THE COMBINATION OF A PROCESSED peptide and a major histocompatibility complex (MHC) molecule comprises the ligand for the T cell receptor (TCR) (*1*). After recognition of peptide-MHC complexes, the TCR transduces signals, resulting in the production of cytokines and in the entry of the cell into a proliferative cycle (*2*). As secreted cytokines execute many of the functions ascribed to $CD4^+$ Th cells, a proliferative response of the Th cell may be unnecessary for an immune response (*3–6*). To examine whether T cell cytokine production inevitably leads to proliferation, we have used a cloned Th cell. Unfortunately, cloned Th cells are selected for their in vitro proliferative response to antigen. To circumvent this problem, we systematically replaced amino acids in an immunogenic peptide, and these altered TCR ligands were then tested for Th cell activation. The change of a single amino acid modifies the TCR ligand so that cytokine production disassociates from proliferation.

Murine $CD4^+$ T cells that react to the β^{dminor} chain of murine Hb respond to the peptide fragment containing amino acids 64 to 76 (*7, 8*). The T helper clone (2.102) used in this study secreted interleukin-4 (IL-4) but not IL-2 or interferon-γ after stimulation and so displays the T helper 2 (Th2) phenotype (*9, 10*). The 2.102 cells proliferated in response to the Hb(64-76) peptide and antigen presenting cells (APCs) (Fig. 1A). When single, conservative amino acid substitutions were introduced into the peptide at positions 69 through 76, a range of effects on proliferation were observed (Table 1). Substituted peptides such as Gln72–Hb(64-76) and Asp73–Hb(64-76) were unable to induce a proliferative response at peptide concentrations as high as 3200 μM, which is equivalent to 4.7 mg of peptide per milliliter (Fig. 1A). Most conservative amino acid substitutions do not affect the binding of MHC molecules and immunogenic peptides (*11*). All of the substituted peptides used in this study bound to MHC $I\text{-}E^k$ molecules in both a functional competition assay and direct binding analysis (*12*).

As an alternative assay for T cell activation, the secretion of IL-4 was measured after stimulation of 2.102 cells with the Hb peptides and APCs (Fig. 1B). In agreement with its inability to support a T cell proliferative response, the Gln72–Hb(64-76) peptide was unable to induce IL-4 production. In contrast, the substitution of Asp for Glu at position 73 stimulated cytokine production (Fig. 1B), even though the substitution was incapable of inducing Th cell proliferation. A small shift (tenfold) was seen in the dose curve generated in response to the Asp73–Hb(64-76) peptide. Peptides that caused similar shifts in the cytokine curve such as Arg76–Hb(64-76) caused Th cell proliferation (Fig. 1A). Although it is difficult to quantitate the minimum amount of IL-4 needed for Th cell proliferation, 100 μM of the Hb(64-76) or the substituted Asp73–Hb(64-76) peptide induced similar concentrations of IL-4, and the addition of exogenous IL-4 [recombinant IL-4 (rIL-4) 250 units/ml or an IL-4 containing supernatant >2000 units/ml] did not result in any proliferation of the Asp73–Hb(64-76)– stimulated cells (*12*). Thus, the lack of proliferation of the Asp73–Hb(64-76)–stimulated cells was not due to the manufacture by the T cells of insufficient IL-4 for autocrine growth. This conclusion is also supported by the observation that the supernatants were collected after 24 hours, at which time there was only minimal T cell proliferation and the T cells would have potentially utilized only a small amount of IL-4.

In MHC-restricted T cell–B cell collaboration, B cell activation of Th cells results in the transmission of an unidentified cell-cell signal that enables the B cell to respond to cytokine (*13, 14*). As an assay for cognate T cell–B cell interaction, the panel of substituted Hb(64-76) peptides and the 2.102 cells were used to stimulate B cells. B cell proliferative responses correlated with the production of cytokine by the Th cells (Table 1). The Ser72–Hb(64-76) peptide did not activate IL-4 secretion by the Th cells and failed to cause B cell proliferation, whereas the Asp73–Hb(64-76) peptide supported a B cell proliferative response, inducing Th cell cytokine production in the absence of Th cell proliferation (Fig. 2A). The polyclonal production of immunoglob-

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ulins by B cells stimulated with the 2.102 cells and Hb peptides confirmed the presence of functional T cell help (Fig. 2B). The change of a Glu to an Asp in the immunogenic peptide resulted in the uncoupling of T cell help from T cell proliferation.

Although we have demonstrated the presence of help in the absence of T cell proliferation, expansion of the Th population may be needed for some immune responses. An inducible costimulatory factor such as IL-1 (*4, 15*) would allow selective expansion of the Th cell population. The addition of exogenous IL-1 at the same time as the Asp73–Hb(64-76) peptide resulted in a normal T cell proliferative response (Fig. 3). Altered TCR ligand was sufficient for cytokine production and led to T cell proliferation if an additional costimulatory signal was provided.

In this study, changes in the TCR ligand allowed the separation of Th cell cytokine production from proliferation. The altered ligand may modify the affinity of interactions with the TCR or the avidity of the TCR and associated molecules for APCs. Regardless of the mechanism of action, the TCR response to ligand is not in an all-or-none manner. When a single amino acid is replaced and one methylene group is removed, the TCR only signals for cytokine production. One way to accomplish gradations of activation through the TCR is by coupling the receptor to multiple signaling pathways. Recent evidence indicates that the TCR and the associated CD3 complex can give rise to separate signals in response to different stimuli (*16–18*).

One pathway coupled to the TCR must relate to the costimulatory phenomenon. In our system, T cell cytokine production and proliferation disassociate in the presence of competent APCs. Costimulation is the re-

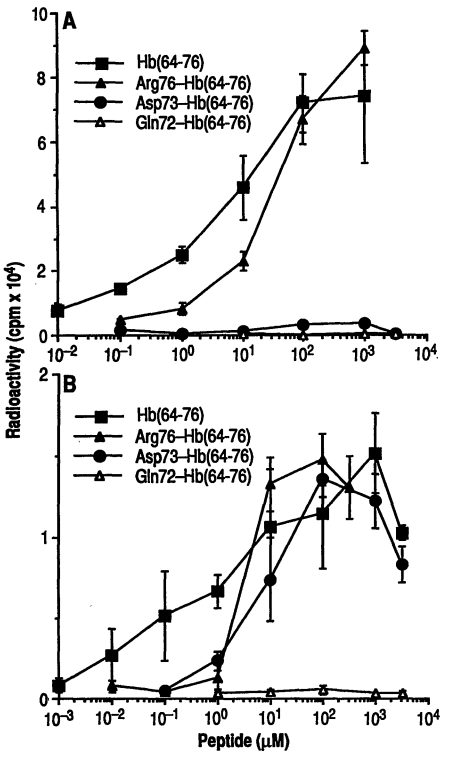

Fig. 1. The response of 2.102 Th2 cells induced by Hb(64-76), Arg76–Hb(64-76), Asp73–Hb(64-76), and Gln72–Hb(64-76) peptides. (**A**) The ability of the selected Hb peptides to induce a proliferative response in 2.102 Th2 cells was assayed by culturing the Th2 cells (10^5) with B10.BR spleen cells (5×10^5) for 72 hours as described (*8*). Tritiated thymidine was added for the final 18 hours of culture. The values represent the mean ± SD of triplicate cultures from one of ten experiments. (**B**) The induction of IL-4 release from 2.102 Th2 cells by culturing the Th2 cells (10^5) with the selected Hb peptides and B10.BR spleen cells (5×10^5) in 200 μl of RPMI medium. After 24 hours, the supernatants (100 μl) were assayed for the presence of IL-4 with the incorporation of tritiated thymidine into the CTLL-2 line sensitive to IL-2 and IL-4 (*8*). The data represent the mean ± SD of triplicate cultures from one of six independent experiments.

Table 1. The response of 2.102 Th2 cells to conservative substitutions in the immunogenic Hb(64-76) peptide. The Hb(64-76) peptide, GKKVITAFNEGLK (*23*), was synthesized and amino acid substitutions were introduced as described (*24*). After purification by high-performance liquid chromatography, the amino acid composition of the peptides was confirmed with an amino acid analyzer. The "–" in the sequence indicates identity with the native Hb(64-76) sequence. T cell proliferation, T cell lymphokine production, and B cell help were assessed as described in Figs. 1, 2, and 3, respectively; the negative sign denotes no detected response at peptide concentrations up to 1000 μM.

Peptide designation	Peptide sequence													T cell proliferation	IL-4 production	B cell help
	64	65	66	67	68	69	70	71	72	73	74	75	76			
Hb 64-76	G	K	K	V	I	T	A	F	N	E	G	L	K	+	+	+
Ser69–Hb(64-76)	–	–	–	–	–	S	–	–	–	–	–	–	–	–	–	–
Ser70–Hb(64-76)	–	–	–	–	–	–	S	–	–	–	–	–	–	+	+	+
Tyr71–Hb(64-76)	–	–	–	–	–	–	–	Y	–	–	–	–	–	+	+	+
Ser72–Hb(64-76)	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–
Gln72–Hb(64-76)	–	–	–	–	–	–	–	–	Q	–	–	–	–	–	–	–
Asp73–Hb(64-76)	–	–	–	–	–	–	–	–	–	D	–	–	–	–	+	+
Ala74–Hb(64-76)	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–
Ile75–Hb(64-76)	–	–	–	–	–	–	–	–	–	–	–	I	–	+	+	+
Arg76–Hb(64-76)	–	–	–	–	–	–	–	–	–	–	–	–	R	+	+	+

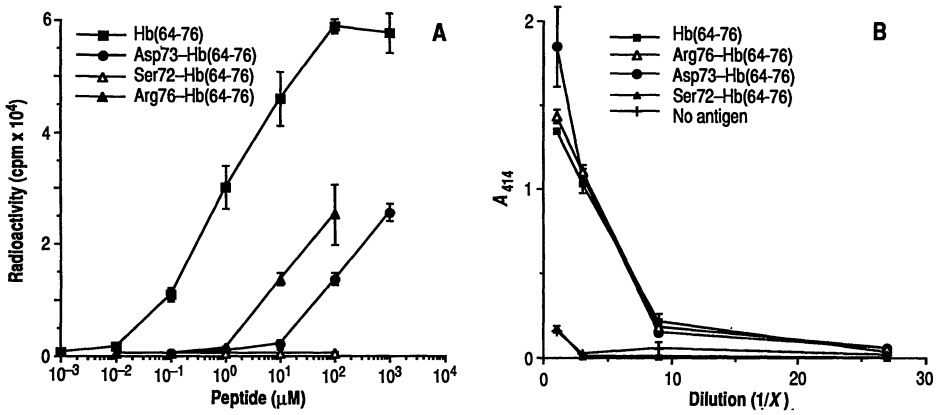

Fig. 2. Analysis of B cell help by 2.102 Th2 cells and Hb(64-76), Arg76–Hb(64-76), Asp73–Hb(64-76), and Ser72–Hb(64-76) peptides. (**A**) The proliferative response of B cells induced by 2.102 Th2 cells and Hb peptides was measured by culturing B10.BR B cells (10^5) with mitomycin-treated 2.102 Th2 cells (10^4) and Hb peptides for 72 hours in 200 μl of RPMI medium. Tritiated thymidine was added to the cultures for the final 18 hours. Control B cell wells stimulated with lipopolysaccharide (20 μg/ml) induced 55,000 cpm. No proliferation (300 cpm) was detected from the mitomycin-treated Th2 cells in response to APCs and Hb(64-76) (10 μM). We purified B10.BR B cells by depleting spleen cells of T cells with a cocktail of monoclonal antibodies and Low-tox rabbit complement (Cedarlane Laboratories) as described (7). B cells were then washed and passed over Ficoll-Paque (Pharmacia) to remove dead cells. Results are reported as the mean ± SD of triplicate cultures. (**B**) Detection of antibody production by B10.BR B cells induced by 2.102 Th2 cells and Hb peptides. B10.BR B cells (10^6) were cultured with 2.102 Th2 cells (10^5) and 100 μM of Hb peptides for 7 days in 1 ml of RPMI medium. Supernatants were collected and assayed for binding to plates coated with Hb (100 μl of 20 μg/ml). The presence of antibody was detected with peroxidase-conjugated goat antibodies to mouse immunoglobulin G and κ light chains (Tago, Burlingame, California); absorbance was read at 414 nm. Results are reported as the mean ± SD of duplicate cultures.

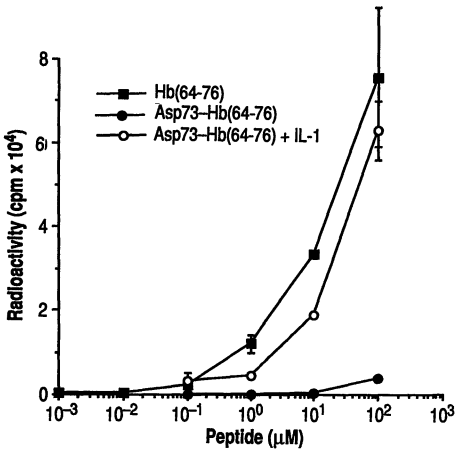

Fig. 3. The Asp73–Hb(64-76) peptide and exogenous IL-1 costimulate the 2.102 Th2 cells to proliferate. The 2.102 Th2 cells (10^5) were cultured with APCs, peptide, and tritiated thymidine as described in Fig. 1. Murine rIL-1β (100 pg/ml) was added to additional wells containing the Asp73–Hb(64-76) peptide and APCs. One of three experiments is shown. In this experiment, 3900 cpm were recorded from the wells containing rIL-1, APCs, and Th2. Addition of rIL-1 to cultures containing the 2.102 Th2 cells and monoclonal antibody to CD3 (145-2C11) in the absence of APCs caused the T cells to proliferate (53,300 cpm). Results are reported as the mean ± SD of triplicate cultures.

quired signal, in addition to recognition of antigen by the TCR, that leads to T cell proliferation (*19, 20*). The altered ligand apparently modifies the interaction between T cell and APC such that proper activation of the APC (*21*) or the reception of signals by the Th cell does not occur (*20*). If costimulation depends on the strength of interaction between TCR and ligand, then activation of T cell cytokine production may be more permissive than expansion of Th cells by proliferation. When an immune response to antigen is evaluated, it may be necessary to consider functional aspects of T cell activation, such as help, because proliferation may not properly reflect the entire response. Differences between the antibody and T cell proliferative responses have been seen with other antigens (*6, 22*). In one case (*6*), changing the MHC molecule and possibly the TCR ligand led to separate responses. Whereas those studies used bulk populations of T cells, in our study it was possible to dissociate the responses of the TCR of a cloned population of Th cells.

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Induction of *Plasmodium falciparum* Transmission-Blocking Antibodies by Recombinant Vaccinia Virus

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Many candidate antigens of malaria vaccines have limited immunological recognition. One exception is Pfs25, a cysteine-rich, 25-kilodalton sexual stage surface protein of *Plasmodium falciparum*. Pfs25 is a target of monoclonal antibodies that block transmission of malaria from vertebrate host to mosquito vector. The surface of mammalian cells infected with a recombinant vaccinia virus that expressed Pfs25 specifically bound transmission-blocking monoclonal antibodies. Furthermore, major histocompatibility complex–disparate congenic mouse strains immunized with recombinant Pfs25 elicited transmission-blocking antibodies, demonstrating that the capacity to develop transmission-blocking antibodies is not genetically restricted in mice. Live recombinant viruses may provide an inexpensive, easily administered alternative to subunit vaccines prepared from purified recombinant proteins to block transmission of malaria in developing countries.

THE SPREAD OF MALARIA CONTINues, despite intensive chemotherapeutic intervention and vector control campaigns. One of several alternatives currently under investigation to slow or reverse the increasing epidemic of malaria is a transmission-blocking vaccine (*1*). Transmission of *P. falciparum* from host to mosquito vector can be blocked by monoclonal antibodies (MAbs) to a 25-kD sexual stage surface protein, Pfs25, expressed on zygotes and ookinetes (*2*). The gene encoding Pfs25 has been cloned (*3*). The deduced amino acid sequence revealed a striking feature: the presence of four tandem epidermal growth factor (EGF)–like domains. EGF-like domains are cysteine-rich and depend on proper disulfide bond formation for structural integrity (*4*). It is not surprising, therefore, that, of the MAbs known to block transmission, none recognize the reduced Pfs25 antigen (*2, 5*), suggesting that, for at least some of the blocking epitopes, disulfide bonds are involved in creating proper conformation.

A subunit vaccine for controlling endemic malaria in developing countries must induce high, long-lasting antibody titers and must be produced in large amounts at the lowest possible cost. Bacteria or yeast provide a simple means of recombinant protein expression that is inexpensive, if the recombinant products can be easily purified and are immunologically effective. Live attenuated viruses, such as vaccinia or adenovirus, are an attractive alternative because they are inexpensive to produce and are easily transported and administered.

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