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In vivo cytotoxic T-lymphocyte induction may take place *via* CD8+ T helper lymphocytes

J.J. Lasarte, P. Sarobe, J. Prieto and F. Borrás-Cuesta (*)

Universidad de Navarra, Facultad de Medicina, Departamento de Medicina Interna, Apartado 273, 31080 Pamplona (Spain)

SUMMARY

Immunization of mice with peptide constructs, consisting of a determinant recognized by T cytotoxic cells colinearly linked to a determinant recognized by T helper cells (TD_c-TD_h) was able to induce cytotoxic T lymphocytes *in vivo*. Interestingly, this induction could be achieved in the absence of adjuvant in non-depleted as well as in $CD4^+$-cell-depleted BALB/c mice. In the latter case, induction took place simultaneously with the activation of $CD8^+$ T helper cells specific for a TD_h contained within the sequence of the TD_c RIQRGPGRAFVTIGK from the immunodominant V3 loop of HIV1 gp120. The possible implications of these findings in HIV infection and AIDS disease are discussed.

Key-words: HIV, T lymphocyte, AIDS, IL2, Cytotoxicity; Induction, $CD8^+$, T helper cells, Determinants, HIV1 gp120, V3 domain, Peptide construct, Mouse, MHC, APC, CTL, Cancer therapy, Vaccine design.

INTRODUCTION

Cytotoxic T lymphocytes (CTL) play an important role in the control of viral infections (Yap *et al.*, 1978; Zinkernagel *et al.*, 1979) and tumour cell growth (Kast *et al.*, 1989; Greenberg, 1991). For these reasons, the understanding of how CTL are induced *in vivo* is of paramount importance both for vaccine design and cancer therapy.

CTL are usually induced following infection with a virus. Thus, cellular enzymes from infected cells process viral proteins into peptides. Some of these peptides, following association with MHC class I molecules are presented at the surface of the infected cell, which after recognition by a specific T-cell receptor leads to CTL activation (Townsend *et al.*, 1989). Immunization with inactivated virus or viral proteins does not usually induce CTL (Moore *et al.*, 1988). This failure is probably due to lack of internalization of the antigen in the cytoplasm of the APC, where endogenous MHC class I processing takes place (Gething *et al.*, 1978; Bangham *et al.*, 1985; Townsend *et al.*, 1986). Since presentation of peptides linked to class I molecules is an essential step for CTL induction, several groups have developed different immunization strategies to achieve CTL induction *in vivo* using peptides

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(*) Corresponding author.

emulsified in Freund's adjuvants (Aichele *et al.*, 1990; Schulz *et al.*, 1991; Fayole *et al.*, 1991; Gao *et al.*, 1991; Hart *et al.*, 1991; Kast *et al.*, 1991), Iscoms (Takahashi *et al.*, 1990b), Qss (Shirai *et al.*, 1994), peptides encapsulated in liposomes (Watari *et al.*, 1987), protein digests or synthetic peptide constructs in the absence of adjuvants (Ishioka *et al.*, 1987; Carbone *et al.*, 1989; Lasarte *et al.*, 1992).

Besides the need for internalization of the antigen in the cell to gain access to the class I processing pathway, it has been reported that the helper activity (IL2 production) mediated by $CD4^+$ cells is required to generate CTL *in vivo* (Keene *et al.*, 1982; Leist *et al.*, 1987, 1989). This suggests that induction of CTL by a peptide requires that both TD_h and TD_c moieties are contained in the peptide. Thus, Fayole *et al.* (1991) and Gao *et al.* (1991) have found that, after depleting mice of $CD4^+$ cells *in vivo*, no CTL activity could be induced by immunization with peptides. However, in other studies using live virus (Buller *et al.*, 1987; Moskophidis *et al.*, 1987; Ahmed *et al.*, 1988; Mizuochi *et al.*, 1989; Allan *et al.*, 1990) or synthetic peptides (Vasilakos *et al.*, 1993), *in vivo* depletion of $CD4^+$ cells did not significantly decrease CTL induction. These results suggested that there might be two pathways for CTL induction, one dependent on and another independent of $CD4^+$ T cells (Fayole *et al.*, 1991).

We provide experimental evidence that CTL can be induced *in vivo* following immunization of mice with peptide construct of the type TD_c-TD_h in the absence of adjuvant. Moreover, this induction was achieved in non-depleted as well as $CD4^+$-cell-depleted BALB/c mice. In the latter case, CTL induction took place simultaneously with the activation of $CD8^+$ T helper cells, which are specific for a TD_h contained within the sequence of RIQRGPGRAFVTIGK, the region from the hypervariable V3 loop from HIV1 that also encompasses the TD_c moiety used in the present study. These experiments show that CTL induction with peptides may indeed take place via two different pathways: (i) a pathway where the T-cell help is provided by $CD4^+$ T helper cells and (ii) a pathway independent of $CD4^+$ T cells, where the T-cell help is provided by $CD8^+$ T helper cells primed by the peptide.

MATERIALS AND METHODS

Peptide synthesis

Peptides were synthesized by the solid phase method of Merrifield (1963) using the Fmoc alternative (Atherton *et al.*, 1989). The synthesis was done manually and the ninhydrin test (Kaiser *et al.*, 1970) was used to monitor every step. Couplings were repeated if necessary until a negative ninhydrin test was attained. Peptides were purified by high-performance liquid chromatography on a reverse-phase C18 column.

Mice

Female BALB/c and B10A mice, 4-6 weeks old, were purchased from Panlab (Barcelona, Spain).

Induction of CTL

Groups of three female mice 4-weeks old were immunized three times at days 0, 7 and 14 by i.p. injection of 100 μg of peptide construct dissolved in 0.5 ml of RPMI-1640 medium. At day 21, mice were killed and spleen cells removed and homogenized. Cells were harvested and cultured *in vitro* in the presence of 5 μg/ml of RIQRGPGRAFVTIGK peptide as described by Ishioka *et al.* (1987). Cytolytic activity was measured 5 days after initial culture. The assays were done in triplicate.

Anti-CD4 and anti-CD8 monoclonal antibodies

The L3T4 (CD4)-specific rat anti-mouse hybridoma GK1-5 and the CD8-specific rat anti-mouse hybridoma H35.17.2 were used to obtain anti-CD4 and anti-CD8 antibodies. Ascitic fluid was obtained from nude mice pristane-primed and injected with 10^6

APC = antigen-presenting cell.
CTL = cytotoxic T lymphocyte.
TD_c = determinant recognized by cytotoxic T cells.
TD_h = determinant recognized by helper T cells.
IL2 = interleukin-2.
i.p. = intraperitoneal.

hybridoma cells. Antibodies were prepared by precipitation with ammonium sulphate and dialysed against phosphate buffered saline. The protein concentration was assessed by measurement of the OD at 280 nm.

Depletion of CD4$^+$ and CD8$^+$ cells *in vivo*

Mice were depleted of CD4$^+$ or CD8$^+$ cells by i.p. injection of 300 µg of anti-CD4 or anti-CD8 antibodies, respectively, on days −1, 0, 1, 6, 7, 8, 13, 14 and 15 (Fayole *et al.*, 1991). The efficiency of the depletion was assessed by flow cytometry on day 21.

IL2 production assay

Spleen cells were plated at 1×10^6 cells per well in a final volume of 200 µl of culture medium in the presence or absence of different dilutions of peptide. Supernatants (50 µl) were removed 24 h later and IL2 content was measured using a CTL.L bioassay as already described (Lai *et al.*, 1987).

Cytotoxic activity

CTL activity was measured using the conventional cytotoxicity assay (Ishioka *et al.*, 1987). Target P815 cells (H-2d-restricted) or EL-4 (H-2b-restricted) previously incubated with 5 µg/ml of relevant peptide were used. The assays were done in triplicate; the spontaneous release was in all cases below 20% of total release.

RESULTS

Induction of CTL in BALB/c mice using synthetic peptide constructs

We previously reported (Lasarte *et al.*, 1992) that a CTL response could be induced *in vivo* by immunization with the peptide construct *RIQRGPGRAFVTIGK*KQIINMWQEVGKAMYA, hereafter referred to as *RIQ*-KQI, containing a TD_c (in italics) and a TD_h (KQI) from HIV1 gp160 (Takahashi *et al.*, 1988; Cease *et al.*, 1987). To expand these results we carried out similar immunization experiments with another peptide construct *RIQRGPGRAFVTIGK*FISEAIIHVLHSR (*RIQ*-FIS) containing the same TD_c but a different TD_h (FIS, residues 106-118 from sperm whale myoglobin (Cease *et al.*, 1986)). Tables I and II show that peptides *RIQ*-

Table I. Characterization of the cytotoxic and helper responses induced in non-depleted and CD4$^+$- or CD8$^+$-depleted BALB/c mice following immunization with *RIQ*-FIS.

			CTL activity			IL2 production			
			% specific lysis			Restimulated with			
In vivo treatment	*In vitro* treatment	E:T ratio	Pulsed with *RIQ*	Unpulsed	Peptide conc. (µg/ml)	*RIQ*-FIS	*RIQ*	FIS	None
None	None	50	30	8	50	31,222	3,459	2,317	584
		16	17.5	5	10	18,994	718	531	
	+anti-CD4				50	4,240	933	300	426
					10	2,419	553	322	
	+anti-CD8				50	11,379	516	572	444
					10	6,911	669	375	
Anti-CD4	None	50	43	12	50	20,379	17,944	759	412
		16	36	6	10	11,968	10,687	713	
	+anti-CD8				50	8,310	727	363	442
					10	6,438	764	405	
Anti-CD8	None	50	2	0.5	50	15,629	1,644	2,730	779
		16	3	0	10	10,197	1,128	1,453	
	+anti-CD4				50	4,080	453	355	355
					10	1,638	439	463	

CTL activity is expressed as % of specific lysis of P815 target cells. IL2 production is expressed as incorporation of 3 H-thymidine in cpm by CTL.L cells (see "Materials and Methods"). This production was measured in the absence or in the presence of 100 µg/ml of anti-CD4 or anti-CD8 antibodies during *in vitro* restimulations with the peptides shown.

Table II. Characterization of the cytotoxic and helper responses induced in non-depleted and CD4+- or CD8+-depleted BALB/c mice following immunization with *RIQ*-KQI.

		CTL activity			IL2 production				
			% specific lysis			Restimulated with			
In vivo treatment	*In vitro* treatment	E:T ratio	Pulsed with *RIQ*	Unpulsed	Peptide conc. (μg/ml)	*RIQ*-KQI	*RIQ*	KQI	None
None	None	50	58	2	50	5,389	1,480	3,753	2,469
		16	36	0	10	3,932	1,746	2,602	
	+anti-CD4				50	678	400	722	360
					10	477	335	446	
	+anti-CD8				50	3,168	1,550	2,730	1,355
					10	2,503	2,211	3,148	
Anti-CD4	None	50	59	20	50	11,894	21,097	804	604
		16	32	6	10	9,661	15,003	804	
	+anti-CD8				50	881	1,201	393	239
					10	687	788	578	
Anti-CD8	None	50	0	1	50	1,602	1,014	715	1,068
		16	2	1	10	2,349	993	916	
	+anti-CD4				50	451	722	632	903
					10	451	1,083	812	

Experimental conditions as in table I.

FIS and *RIQ*-KQI were able to induce a CTL response against P815 target cells preincubated with *RIQ*. By contrast, free *RIQ* was unable to induce this response (table III). Induced CTL presented the surface phenotype $CD4^-CD8^+$ as assessed by flow cytometry and by cytotoxic assays in the presence of anti-CD4 or anti-CD8 antibodies plus complement. Induced CTL were able to lyse P815 preincubated with RIQ, but unable to lyse EL-4 cells, showing that they were $H\text{-}2^d$-restricted (data not shown).

CTL induction following depletion of $CD4^+$ or $CD8^+$ cells in BALB/c mice

To study the role of $CD4^+$ and $CD8^+$ cells in CTL induction following immunization with *RIQ*-FIS and *RIQ*-KQI, mice were depleted of $CD4^+$ or $CD8^+$ cells by i.p. injection of anti-CD4 or anti-CD8 antibodies, respectively, as described in "Materials and Methods". At day 21, mice were killed and the spleen cells removed and homogenized. Analysis of an aliquot of these cells by flow cytometry showed that depletion of $CD4^+$ or $CD8^+$ cells was, in all cases, at least 95% (data not shown). Following *in vitro* restimulation of the remaining spleen cells, under the conditions specified in "Materials and Methods", CTL activity was measured. Tables I and II show that, both in *RIQ*-FIS and *RIQ*-KQI immunized mice which had been depleted of $CD4^+$ cells, CTL activity against P815 target cells incubated with *RIQ* was not lost, in contrast with the complete abrogation of activity observed in mice depleted of $CD8^+$ cells.

Effect of anti-IL2 receptor antibodies on CTL activity during *in vitro* restimulation

In order to assess whether CTL induction was IL2-dependent during restimulations, the IL2 receptor was blocked by adding anti-IL2 receptor antibodies. Figure 1 shows that following the addition of these antibodies to spleen cells from mice immunized with *RIQ*-FIS, CTL induction was blocked, proving that the presence of IL2 is

Table III. Characterization of the cytotoxic and helper responses induced in non-depleted or CD4$^+$-cell-depleted BALB/c mice following immunization with *RIQ*.

			CTL activity		IL2 production		
			% specific lysis		Restimulated with		
In vivo treatment	*In vitro* treatment	E:T ratio	Pulsed with *RIQ*	Unpulsed	Peptide conc. (μg/ml)	*RIQ*	None
None	None	81	24	20	50	932	568
		27	11.2	10.9	10	1,197	
	+anti-CD4				50	600	538
					10	323	
	+anti-CD8				50	1,524	584
					10	1,052	
Anti-CD4	None	81	9.6	4.7	50	602	208
		27	1.0	0.5	10	381	
	+anti-CD8				50	483	305
					10	480	

Experimental conditions as in table I.

essential for *in vitro* expansion of the CTL induced *in vivo*.

Effect of anti-CD4$^+$ or anti-CD8$^+$ addition on CTL activity during *in vitro* restimulation of cells from non-depleted immunized mice

To study the effect of CD4$^+$ and CD8$^+$ cells during *in vitro* restimulation of spleen cells from *RIQ*-KQI immunized BALB/c mice (non-depleted of CD4$^+$ or CD8$^+$ cells), we added anti-CD4 or anti-CD8 antibodies (100 μg/ml, final concentration, of the corresponding antibody) to the *in vitro* restimulation medium. Table IV shows that both anti-CD4$^+$ and anti-CD8$^+$ antibodies abrogated CTL activity.

IL2 production

IL2 production was measured in parallel with CTL induction from cells induced following immunization with peptides.

The results of these experiments are shown in tables I, II and III. Cells from normal mice (non-depleted of either CD4$^+$ or CD8$^+$ cells) immunized with *RIQ*-FIS produced high levels of IL2 in the presence of *RIQ*-FIS (table I). This production was substantially blocked after adding anti-CD4 antibodies *in vitro* and, to a lesser extent, following the addition of anti-CD8 antibodies. It is interesting to note that IL2 production was mainly associated with *RIQ*-FIS, and that free *RIQ* and free FIS were much less active.

In the group of non-depleted mice immunized with *RIQ*-KQI (table II), it can be seen that restimulation with *RIQ*-KQI or with free KQI induced IL2 production, which was only twice that attained following restimulation without peptide. Mice immunized with *RIQ*, depleted or non-depleted of CD4$^+$ cells, were unable to induce IL2 production (table III).

Mice depleted of CD4$^+$ cells *in vivo* and immunized with *RIQ*-FIS (table I) did not lose the ability to induce IL2 production following *in vitro* restimulation with *RIQ*-FIS. It is interesting to note that here *RIQ* was responsible for this induction. Also, IL2 production could be completely blocked by anti-CD8 antibodies if restimulations were carried out with *RIQ*. A substantial but incomplete decrease was

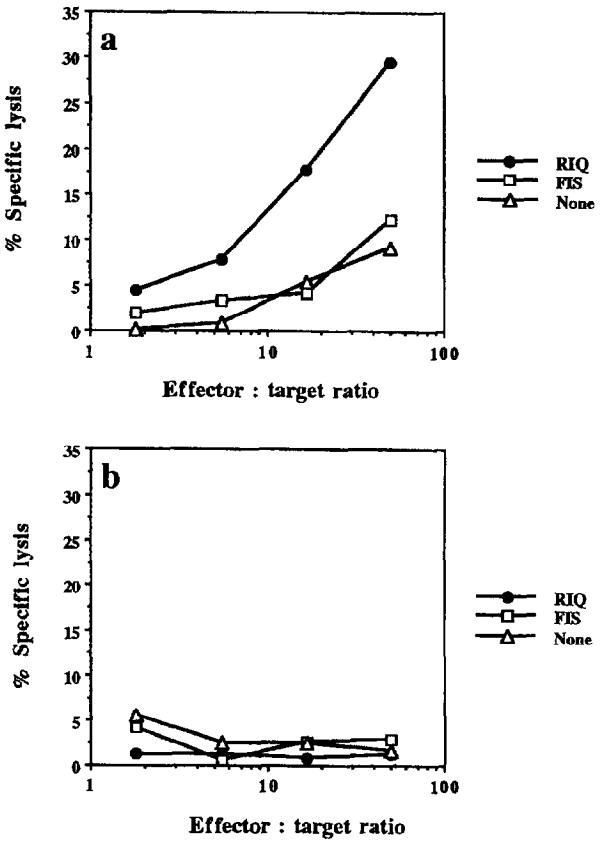

Fig. 1. Effect of anti-IL2-receptor antibodies on CTL activity during *in vitro* restimulation.

Spleen cells of BALB/c mice immunized with *RIQ*-FIS were restimulated *in vitro* with RIQ (a) in the absence or (b) in the presence of anti-IL2-receptor antibodies. P815 target cells were preincubated with the antigens shown.

observed when restimulation was carried out with *RIQ*-FIS.

Mice depleted of CD4$^+$ cells *in vivo* and immunized with *RIQ*-KQI (table II), showed a high level of IL2 production in the presence of *RIQ* peptide. This induction could be blocked almost completely by anti-CD8 antibodies, when *RIQ*-KQI or *RIQ* was used in the *in vitro* restimulations.

Mice depleted of CD8$^+$ cells *in vivo* and immunized with *RIQ*-FIS (table I) induced IL2 production mainly when restimulated with *RIQ*-FIS. This induction was substantially decreased after adding anti-CD4 antibodies during *in vitro*

Table IV. Effect of anti-CD4 or anti-CD8 antibody addition on CTL activity during *in vitro* restimulation of cells from non-depleted BALB/c mice immunized with *RIQ*-KQI.

		CTL activity % specific lysis	
In vitro treatment	E:T ratio	Pulsed with *RIQ*	Unpulsed
None	50	74	46
	16.6	38	21
+anti-CD4	50	11	4
	16.6	2	1
+anti-CD8	50	6	2
	16.6	1	3

Spleen cells were restimulated with *RIQ* in the absence or in the presence of 100 μg/ml of anti-CD4 or anti-CD8 antibodies.

restimulation with peptide. Much poorer IL2 production was observed if restimulations were carried out with *RIQ* or FIS. This production could be completely blocked after adding anti-CD4 antibodies. Similarly, when mice depleted of CD8$^+$ cells were immunized with *RIQ*-KQI (table II), *RIQ*-mediated IL2 production was lost, in contrast with the results obtained with mice depleted of CD4$^+$ cells.

Induction of CTL in B10A mice (IA^k, H-2^d) with *RIQ*-FIS; effect of *in vivo* depletion of CD4$^+$ cells on CTL induction and IL2 production

It has been reported that *RIQ* is a TD_h for CD4$^+$ helper lymphocytes of BALB/c mice (Takahashi *et al.*, 1990a). However, *RIQ* is also recognized as TD_h by CD8$^+$ lymphocytes (tables I and II). In order to confirm the association of this TD_h activity with MHC class I (H-2^d) restriction, we immunized non-depleted and CD4$^+$-cell-depleted B10A mice (IA^k, H-2^d-restricted) with *RIQ*-FIS. These mice share with BALB/c the class I restriction, but differ in their class II restriction. The CTL response as well as the IL2 production in the presence of the different pep-

tides was studied. Table V shows that *RIQ*-FIS induced a significant cytotoxic response only in mice depleted of CD4 cells. IL2 production was also stronger in this group of mice and was mainly due to *RIQ*. This result confirms that the TD_h character of *RIQ* is associated with $CD8^+$ helper cells, and suggests that it is linked to MHC class I molecules.

DISCUSSION

In addition to internalization of the antigen in the cell in order to gain access to the class I processing pathway, activation of a helper response leading to the production of lymphokines is also essential for proliferation and maturation of CTLs (Keene *et al.*, 1982; Leist *et al.*, 1987, 1989).

As was found for two synthetic peptides from LCMV and influenza virus (Fayole *et al.*, 1991; Gao *et al.*, 1991), and as suggested by us for the peptide construct *RIQ*-KQI from HIV1 gp120 (Lasarte *et al.*, 1992), induction of a CTL response with these synthetic peptides requires coactivation of a $CD4^+$ helper response. Moreover, the recent work of Shirai *et al.* (1994) with a similar peptide construct containing *RIQ* and KQI administered in QS21 saponine adjuvant has confirmed that help from $CD4^+$ lymphocytes induced by the peptide construct is required.

The results of IL2 production reported in tables I and II for non-depleted mice are essentially in agreement with the findings discussed above. Indeed, IL2 production by spleen cells from normal BALB/c mice immunized with *RIQ*-FIS or *RIQ*-KQI is dramatically decreased by adding anti-CD4 antibodies during *in vitro* restimulation. Moreover, *in vitro* addition of anti-IL2-receptor antibodies to spleen cells blocks the restimulation of CTL activity (fig. 1). This restimulation is also blocked by the addition of anti-CD4 as well as by anti-CD8 antibodies (table IV). All these observations suggest that $CD4^+$ cells are required to provide T-cell help to $CD8^+$ cytotoxic T cells. However, in some viral models, it has been observed that *in vivo* depletion of $CD4^+$ cells does not abrogate cytotoxic activity (Buller *et al.*, 1987; Moskophidis *et al.*, 1987; Ahmed *et al.*, 1988; Mizuochi *et al.*, 1989; Allan *et al.*, 1990). Also, it has been shown that CTL induction after a viral infection can be achieved in the absence of $CD4^+$ cells when $CD8^+$ cells produce the necessary lymphokines to support this induction (Mizuochi *et al.*, 1989). To explain this apparent discrepancy concerning the role of $CD4^+$ cells in CTL induction, it has been suggested that two different pathways of CTL induction may exist: one dependent on and another independent of $CD4^+$-T-cell help (Fayole *et al.*, 1991).

To study in more detail the role of different cell populations in CTL induction using synthetic peptides, we analysed the effect of *in vivo* $CD4^+$ or $CD8^+$ depletion. These experiments showed

Table V. Characterization of the cytotoxic and helper responses induced in non-depleted or $CD4^+$-depleted B10A mice following immunization with *RIQ*-FIS.

		CTL activity			IL2 production			
		% specific lysis			Restimulated with			
In vivo treatment	E:T ratio	Pulsed with *RIQ*	Unpulsed	Peptide conc. µg/ml	*RIQ*-FIS	*RIQ*	FIS	None
None	25	10.7	6.1	50	2,394	858	820	644
	8.2	3.1	4.1	10	1,966	933	1,235	
Anti-CD4	20	19.0	0.1	50	6,711	4,640	968	950
	6.6	9.3	3.6	10	6,541	3,603	1,600	

Experimental conditions as in table I.

that $CD4^+$ depletion did not abrogate CTL induction (tables I and II). Since lymphokines may be required in this process, we analysed IL2 production by spleen cells from different groups of mice immunized with peptides. As shown in tables I and II for *in vivo* $CD4^+$-depleted mice, *RIQ*-FIS and *RIQ*-KQI constructs induced high levels of IL2. This production was mediated by *RIQ* and could be abrogated by the addition of anti-CD8 antibodies. Moreover, *in vivo* depletion of $CD8^+$ cells abrogated IL2 production linked to *RIQ*. These results show that the *RIQ* moiety contains a $CD8^+$ helper determinant that may be implicated in *in vivo* CTL induction. However, as shown in table III, immunizations of normal or $CD4^+$-depleted BALB/c mice with free *RIQ* were unable to induce measurable CTL responses. This result has also been reported by Berzofsky (1991), Lasarte *et al.* (1992) and Shirai *et al.* (1994). We believe that this might be related to poor internalization of *RIQ* in the cells, thereby preventing their processing via the MHC class I pathway.

Although the efficiency of *in vivo* $CD4^+$ depletion was greater than 95% (as measured by flow cytometry; data not shown), it could be argued that IL2 production linked to *RIQ* in $CD4^+$-depleted mice immunized with *RIQ*-FIS or *RIQ*-KQI may have been due to the remaining $CD4^+$ cells. To show that IL2 production induced by *RIQ* was linked to class I molecules rather than to class II molecules, we immunized B10A mice (IA^k, $H\text{-}2^d$) that share with BALB/c (IA^d, $H\text{-}2^d$) only the class I molecules. Thus, $CD4^+$-depleted B10A mice immunized with *RIQ*-FIS elicited helper and cytotoxic T-cell responses linked to *RIQ* (table V), showing that these responses are associated with $H\text{-}2^d$ class I molecules. It is not clear why $CD4^+$ depletion is required for induction of detectable specific lysis in B10A mice. However, a tentative explanation might be the following: as shown in table V, immunization of normal B10A mice with *RIQ*-FIS did not elicit T-cell help from either $CD4^+$ or $CD8^+$ cells. Thus, as expected, no CTL induction could take place due to the absence of this help. However, since T-cell help from $CD8^+$ cells was elicited after depletion of $CD4^+$ cells, this led to a concomitant induction of CTL. As opposed to B10A mice, non-depleted BALB/c mice immunized with *RIQ*-FIS were able to elicit T-cell help from $CD4^+$ cells, and consequently CTL. However, both in B10A and BALB/c mice, the pathway of CTL induction linked to help from $CD8^+$ cells takes place only after depletion of $CD4^+$ cells (tables I, II and V). A possible interpretation of this result might be that $CD4^+$ cells sequester the antigen, favouring the $CD4^+$-cell-dependent pathway. Another alternative interpretation is that $CD4^+$ cells might exert a suppressor effect on $CD8^+$ cells.

Under normal conditions (non-depleted mice), the induction of CTL seems to take place *via* the help from $CD4^+$ cells. Indeed, as shown in table IV, the CTL activity induced *in vivo* in non-depleted mice can be abrogated by the addition of anti-CD4 antibodies during *in vitro* restimulation. This result is in agreement with that reported by Takahashi *et al.* (1990a) on CTL induction using recombinant vaccinia virus expressing HIV1 gp120.

The appearance of AIDS in HIV-infected patients occurs when the cytotoxic activity and the $CD4^+$ count are low (Gruters *et al.*, 1991). However, the cytotoxic activity seems to remain unchanged for a long period of time, while the $CD4^+$ count decreases progressively (Rowland-Jones and McMichael, 1993). It is tempting to postulate that the cytotoxic pathway independent of $CD4^+$ helper cells that we found in $CD4^+$-depleted mice might also be induced at this stage in HIV-infected patients. This might compensate over time for the loss of $CD4^+$ T-cell help over a certain period of time. Moreover, as shown by Mizuochi *et al.* (1989) for the case of vaccinia virus infection, the activation of CTL *via* the $CD8^+$ T helper pathway decreases progressively with increasing time. Based on the observation made by Moskophidis *et al.* (1993) for LCMV infection, showing that the disappearance of CTL activity is due to clonal deletion by exhaustion associated with high viral levels, it could be postulated that the hypothetical $CD4^+$-independent cytotoxic activity in AIDS might vanish due to this clonal deletion. However, if the $CD4^+$-independent pathway for CTL activation has not been

induced in HIV-infected patients with a low $CD4^+$ count, immunization of patients with peptide constructs like *RIQ*-KQI or *RIQ*-FIS at this stage, might offer some hope of keeping HIV at bay, thus delaying the appearance of AIDS.

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L'induction *in vivo* de lymphocytes T cytotoxiques est possible *via* la cellule T «helper» $CD8^+$

L'immunisation de la souris au moyen de peptides synthétiques ayant un déterminant reconnu par les cellules T auxilliares, TD_c-TD_h, s'avère capable d'induire *in vivo* des lymphocytes T cytotoxiques. Il est notable que cette induction aboutit en l'absence d'adjuvant, que la souris BALB/c soit porteuse ou dépourvue de cellules $CD4^+$; dans ce dernier cas, l'induction a lieu simultanément avec l'activation de cellules T «helper» $CD8^+$ spécifiques du déterminant TD_h contenu dans la séquence RIQRGPGRAFVTIGK correspondant au TD_c de la boucle V3 de la gp120 du VIH (virus de l'immunodéficience humaine). Les possibles implications de ces faits dans l'infection par le VIH et dans le SIDA sont discutées.

Mots-clés: VIH, Lymphocyte T, SIDA, IL2, Cytotoxicité; Induction, $CD8^+$, Cellules T auxilliaires, Déterminants, Glycoprotéine gp120 du VIH1, Domaine V3, Peptides synthétiques, CMH, Souris, APC, CTL, Vaccins, Thérapie anticancéreuse.

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