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Immunomodulation induced by synthetic peptides derived from *Staphylococcus aureus* protein A

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Abstract

Peptides from 10 to 22 amino acids containing sequences encompassed by *Staphylococcus aureus* protein A were synthesized. Some of these peptides, when present in cultures of lymphomononuclear cells from healthy donors or from cancer patients (melanoma, breast carcinoma, non-Hodgkin lymphoma and renal cell carcinoma) promoted: (i) changes in the phenotype of the lymphomononuclear population, (ii) stimulation of monocytes (release of IL-1 and TNF-α), and (iii) an increase in cytotoxicity against K562, Daudi and HT-29 cells. Isolated monocytes responded also to those peptides with a release of IL-1 and TNFα and an increase of cytotoxicity against HT-29 cells. It was found that the active peptides had the following structural pattern: a length of at least 15 amino-acid residues with a proline at position 6, valine, leucine, isoleucine, glycine, alanine or lysine at position 2, and glutamic or aspartic acid at position 11. Replacement of Pro at position 6 with any other residue turned the peptide inactive. Replacement of residues at positions 2 and 11 with amino-acid residues other than those required for activity resulted in compounds with a marked decrease in the immunomodulating properties described, or lacking these properties altogether.

Key words: Immunomodulatory peptide; Cytotoxicity; Tumor necrosis factor α; Interleukin 1; Lymphomononuclear cell; Monocyte; (Human)

1. Introduction

Protein A from *Staphylococcus aureus* (SpA) [1,2] has a wide variety of immunological properties, such as the stimulation of lymphocyte subpopulations, the release of interferon and natural killer activity [3–7]. In general, exogenous antigens require processing by antigen presenting cells (APC) before their association with MHC proteins to form a complex that can be recognized by specific T cell receptors [8,9]. Helper T cells are stimulated by MHC class II molecules which generally present peptides from antigens that have entered B cells or macrophages by endocytosis [10]. T cells do not recognize native protein antigens, unless previously denatured or partially degraded, and subsequently displayed in association with MHC class II (Ia) molecules by APC. The pattern of peptide association with various allelic variants of Ia reflects the pattern of MHC restriction of the immune response [11,12]. The structural requirements for their interactions have been subjected to an intense investigation. Several models have been elaborated to predict T cell epitopes based on either the presence of amphipathic α-helixes [13], amino-acid sequence motifs [14], or structural similarities of MHC core-binding regions on previously identified epitopes [15,16]. However, the binding of an immunodominant peptide to a class II molecule is a necessary, but not sufficient condition to promote an immune response.

We speculated that short synthetic peptides derived from SpA might have similar immunological properties to those exhibited by the native protein, and, if that were the case, that the possible correlation between those properties and the structural features of the active peptides could be established. We show below

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that peptides derived from SpA are indeed biologically active and that their activity seems to be related to a common structural pattern.

2. Materials and methods

Cell source and preparations

Peripheral blood mononuclear cells from healthy donors or from cancer patients, when indicated, were obtained by leukapheresis, using a Fenwall CS-3000 continuous flow separator (Fenwall Laboratories, Deerfield, IL, USA). Vascular access was obtained with a double central venous catheter. Isolation and purification of monocytes was carried out following the technique described by Bøyum [17].

Cell culture conditions

Peripheral blood mononuclear cells were cultivated at $2 \cdot 10^6$ cells/ml in RPMI-1640 medium supplemented with 2.5% of autologous serum, 200 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) in humidified atmosphere with 5% CO_2 at 37°C. Peptides were added at a concentration of 20 $\mu g/10^6$ cells; rIL-2 at 500 $U/10^6$ cells and SpA at 20 $\mu g/10^6$ cells.

Chemical synthesis of the peptides

The synthesis of the various peptides was carried out by the solid-phase method of Merrifield [18], with the Fmoc modification [19].

Surface markers

Immunofluorescence studies were performed with a Coulter Epics flow cytometer (Coulter Electronics, Hialeah, FL). The monoclonal antibodies used in this study were: anti-Leu-4 (CD3), Leu-2a (CD8), Leu-11a (CD16), Leu-19 (CD56), Leu-M3 (CD14) and HLA-DR, obtained from Becton-Dickinson Immunocytometry Systems, (Erembodegen, Belgium) and anti-MAC1 (CD11b) from Immunotech (Marseilles, France). All these antibodies were used as conjugates of fluorescein isothiocyanate (FITC). Each assay was accompanied with its appropriate isotype control. $5 \cdot 10^5$ cells were incubated in 50 μl of PBS-0.1% BSA with the monoclonal antibody during 30 min at 4°C. After incubation the cells were washed twice with cold PBS-0.1% BSA. Cells were then fixed in 500 μl of formaldehyde in 2% PBS. Forward angle (FALS) and 90° light scattering (90° LS) measurements were recorded. 5000 cells were analyzed for each determination. The percentage of positive cells was determined after substraction of non-specific labelling events, which, in turn, were evaluated in each measurement with the corresponding isotype control.

Cytokine determinations

IL-1 and TNFα were measured by the quantitative 'sandwich' enzyme immunoassay technique with commercially available kits (Quantikine, R&D Systems, Minneapolis, USA).

Cytotoxicity assay

The 4 h ^{51}Cr release cytolysis assay [20] was performed with K562 [21] and Daudi [22] cell lines as targets. Target cells $(1 \cdot 10^4)$ were added to serially diluted $((50–5) \cdot 10^4)$ effector cells in RPMI containing 2.5% autologous serum in round-bottomed microtiter plates. All determinations were carried out in triplicate. The percentage lysis was calculated by the formula ((experimental release − spontaneous release)/(maximal cpm incorporated − spontaneous release)) × 100. The spontaneous ^{51}Cr release from cell line targets was always $< 10\%$. Cytotoxicity associated with monocytes was measured following the technique of Meltzer [23] using purified monocytes or lymphomononuclear cells as effector cells, and HT-29 cells from a human adenocarcinoma [24] cultured in monolayer as target cells. HT-29 cells were labelled with 1 mCi/ml of [^{3}H]thymidine when they had a confluence of 50% and incubated at 37°C for 16–18 h. The incubation with the effector cell was carried out at 37°C and 5% CO_2.

3. Results and discussion

Cytotoxicity induced by synthetic peptides derived from SpA

The amino-acid sequence of Cowan I strain staphylococcal protein (SpA) is already well established [1,2]. Peptides consisting of 15 amino acids (14 of them reflecting the sequence of the four Fc-binding regions (regions D, A, B and C of SpA as described by Sjödahl [2]) were synthesized. The extra amino acid, always on the carboxyl end, was valine which was added for synthesis convenience. Table 1 shows the ability of synthetic peptides to induce cytotoxicity against tumor cell lines, Daudi and K562, when lymphomononuclear cells were incubated in their presence. IL-2 was used as a reference control.

A close examination of the peptides reveals that all the active structures with regard to cytotoxicity include a proline residue at position 6 and a glutamic acid residue located at the 11th position. The nature of the amino-acid residues at positions 2 and 3 seems also to be of importance, position 2 being a leucine, isoleucine, valine or a lysine residue, and position 3 preferentially leucine.

These results prompted us to synthesize new peptides introducing amino-acid substitutions in some of the active peptides derived from protein A. Table 2

Table 1
Cytotoxicity induced by synthetic peptides, derived from SpA on lymphomononuclear cells from healthy donors

Peptide No.	Sequence [a]	Position [b]	Cytotoxicity	
			Daudi	K562
1	ADAQQNKFNKDQQS	1–14	–	–
2	QNKFNKDQQSAFYE	5–18	–	–
3	KDQQSAFYEILNMP	10–23	–	–
4	ADNNFNKEQQNAFY	62–75	–	–
5	ADNKFNKEQQNAFY	120–133	–	–
6	FNKDQQSAFYEILN	8–21	–	–
7	FNKEQQNAFYEILN	66–79	–	–
8	FNKEQQNAFYEILH	182–195	–	–
9	QSAFYEILNMPMLN	13–26	–	–
10	QNAFYEILNMPNLN	71–84	–	–
11	QNAFYEILHLPNLN	129–143	–	–
12	QNAFYEILHLPNLT	187–200	–	–
13	EILNMPNLNEEQRN	18–31	+ +	+ +
14	EILHLPNLNEEQRN	134–147	+ +	+ +
15	EILHLPNLTEEQRN	192–205	+ +	+ +
16	MPNLNEEQRNGFIQ	22–35	–	–
17	LPNLNEEQRNGFIQ	138–151	–	–
18	LPNLTEEQRNGFIQ	196–209	–	–
19	EEQRNGFIQSLKDD	27–40	–	–
20	GFIQSLKDDPSQST	32–45	–	–
21	GFIQSLKDDPSQSA	90–103	–	–
22	GFIQSLKDDPSVSK	206–219	–	–
23	SLKDDPSQSTNVLG	36–49	+	+
24	SLKDDPSQSANLLA	94–107	+	+
25	SLKDDPSVSKEILA	210–223	+ +	+ +
26	PSQSTNVLGEAKKL	41–54	–	–
27	PSQSANLLAEAKKL	99–112	–	–
28	PSVSKEILAEAKKL	215–228	–	–
29	NVLGEAKKLNESQA	46–59	–	–
30	NLLAEAKKLNESQA	104–117	–	–
31	NLLAEAKKLNDAQA	162–175	–	–
32	EILAEAKKLNDAQA	220–233	–	–
33	KKLNESQAPKADNN	52–65	–	–
34	KKLNESQAPKADNK	110–123	–	–
35	KKLNDAQAPKADNN	168–181	–	–
36	ESQAPKADNNFNKE	56–69	–	–
37	ESQAPKADNKFNKE	114–127	–	–
38	DAQAPKADNNFNKE	172–185	–	–
39	YEILNMPNLNEEQR	17–30	–	–
40	FYEILNNPNLNEEQ	16–29	–	–
41	QSLKDDPSVSKEIL	209–222	–	–
42	IQSLKDDPSVSKEI	208–221	–	–
43	FIQSLKDDPSVSKE	207–220	–	–
44	FYEILHLPNLNEEQ	132–145	–	–
45	YEILHLPNLTEEQR	191–204	–	–
46	FYEILHLPNLTEEQ	190–203	–	–

Peptides were scored for expressed cytotoxicity as follows: + +, good inducers; +, intermediate inducers; –, non-inducers. Peptide concentration was always 20 $\mu g/10^6$ cells. rIL-2 at a concentration of 500 $U/10^6$ cells was used as a reference control. Cytotoxicity was determined after 7 days of incubation.
[a] An extra valine residue, not shown, was added at the terminus for synthesis convenience. [b] Numbering of the amino-acid residue taken from Sjödahl [2].

shows that the replacement of proline by alanine or glycine in the active peptides 13, 14, 15 or 25 resulted in inactive peptides. This confirmed the requirement of proline at the indicated position (peptides 47–53). However, the replacement at position 6 by proline in inactive peptides 54 and 55 was unable to render them active. Some other modifications were also introduced in inactive peptides. In peptide 29 (NVLGEAKKLNESQA) Glu at position 5 was replaced by Ala, and Ala at position 6 by Pro. The resulting peptide (peptide 56) was very active with regard to antitumor cytotoxicity against Daudi and K562 cells. Table 3 shows the cytotoxicity induced on lymphomononuclear cells by synthetic peptides resulting from modifications of peptide 56. The results showed that for a peptide of 15 amino acids, the requirements for activity were: proline at position 6, glutamic acid at position 11, and leucine, isoleucine or valine at position 2. With the exception of peptide 25, in all active peptides tested, position 3 was always leucine. The peptides resulting from the replacement of Val at position 2 of peptide 56 by Lys or a hydrophobic amino-acid residue were still active. However, this activity was rather low. The activity was also greatly reduced if Glu at position 11 was replaced by Asp. The replacement of an amino-acid residue at any position either to the right or to the left of proline at position 6 by proline resulted in inactive peptides.

The non-Fc-binding region E of SpA contains also a sequence fulfilling the structural pattern of active peptides. The synthetic peptide QVLNMPNLNADQRNV, which contains 14 amino-acid residues of such a sequence plus an extra valine at the C end, also induced cytotoxicity.

The length of the peptide was also tested. Extended peptides containing the structure of an active peptide were also active. This was the case of peptides DQQSAFYEILNMPNLNEEQR (peptide 81, sequence 11–30 in SpA) and NGFIQSLKDDPSVSKEILAEAK (peptide 82, sequence 205–226 in SpA). However, peptides shortened from either the amino or the carboxyl end were inactive (data not shown).

Table 2
Effect of single amino-acid substitutions at position 6 on the cytotoxic activity of synthetic peptides

Peptide No.	Sequence [a]	Derived from wild peptide	Cytotoxicity	
			Daudi	K562
47	EILNM**A**NLNEEQRNV	13 (P → A)	–	–
48	EILHL**A**NLNEEQRNV	14 (P → A)	–	–
49	EILHL**A**NLTEEQRNV	15 (P → A)	–	–
50	EILNM**G**NLLEEQRNV	13 (P → G)	–	–
51	SLKDD**G**SVSKEILAV	25 (P → G)	–	–
52	EILHL**G**NLNEEQRNV	14 (P → G)	–	–
53	EILHL**G**NLTEEQRNV	15 (P → G)	–	–
54	YEILH**P**PNLNEEQRV	45 (L → P)	–	–
55	ADAQQ**P**KFNKDQQSV	1 (N → P)	–	–

Peptide concentration was always 20 $\mu g/10^6$ cells. rIL-2 at a concentration of 500 $U/10^6$ cells was used as a reference control. Cytotoxicity was determined after 7 days of incubation.
[a] In bold: amino-acid replacement at position 6 of the wild peptide.

Table 3
Cytotoxicity induced on lymphomononuclear cells from healthy donors following amino-acid replacement in the sequence of peptide 56

Peptide No.	Sequence [a]	Cytotoxicity	
		Daudi	K562
56	NVLGAPKKLNESQAV	++	++
57	NVLPAPKKLNESQAV	+	+
58	NVLGAPKKPNESQAV	+	+
59	NTLGAPKKLNESQAV	+	+
60	NCLGAPKKLNESQAV	+	+
61	NKLGAPKKLNESQAV	+	+
62	NFLGAPKKLNESQAV	−	−
63	NMLGAPKKLNESQAV	−	−
64	NQLGAPKKLNESQAV	−	−
65	NWLGAPKKLNESQAV	−	−
66	NSLGAPKKLNESQAV	−	−
67	NYLGAPKKLNESQAV	−	−
68	NALGAPKKLNESQAV	+	+
69	NGLGAPKKLNESQAV	+	+
70	NVLGAAKKLNESQAV	−	−
71	NVLGAGKKLNESQAV	−	−
72	NVLGAPKLLNKSQAV	+	−
73	NVLGAPKKLNVSQAV	−	−
74	NVLGAPKKLNDSQAV	+	+
75	NVLGAPKKLNTSQAV	−	−
76	NVLGAPKKLNSSQAV	−	−
77	NVLGMPKKLNESQAV	+	+
78	NVLGEPKKLNESQAV	−	−
79	NKLGAPKKLNKSQAV	+	+
80	NELGAPKKLNVSQAV	−	−

Peptide concentration was always 20 $\mu g/10^6$ cells. rIL-2 at a concentration of 500 $U/10^6$ cells was used as a reference control. Cytotoxicity was determined after 7 days of incubation.
[a] In bold: amino-acid replacement respect to wild peptide 56.

Table 4
Peptide-induced cytotoxic activity against K562, Daudi and HT-29 target cells

Product tested	Cytotoxic activity		
	(% chromium release)		(% [^{3}H]thymidine release)
	Daudi	K562	HT-29
Medium	9±3	19±5	27±5
Peptide No. 56	68±6	63±8	62±6
Peptide No. 13	62±8	62±7	59±5
Peptide No. 25	58±9	59±9	57±5
Peptide No. 81	56±6	58±7	58±6
Inactive peptide No. 2	10±4	12±8	29±4
rIL-2	80±9	70±6	n.d.
SpA	74±9	68±7	67±5
IFNγ	n.d.	n.d.	70±5

Lymphomononuclear cells were obtained from five different healthy donors. Results represent mean±S.E. SpA concentration was 20 $\mu g/10^6$ cells. Peptide concentration was always 20 $\mu g/10^6$ cells. rIL-2 (500 $U/10^6$ cells) or IFNγ (100 $U/10^6$ cells) were used as reference controls. Cytotoxicity was determined after 7 days of incubation. Effector-to-target cell ratio was 50:1 for K562 and Daudi cells, and 20:1 for HT-29 cells. n.d., not determined.

Table 4 shows the cytotoxicity induced by the most active peptides as well as that induced by SpA or IL-2. The cytotoxicity induced by the synthetic peptides against K562 cells was similar to that induced by IL-2 or SpA. The activity against Daudi cells was slightly lower in the case of the synthetic peptides. SpA and active peptides also induced cytotoxicity against HT-29 cells, reaching values similar to those obtained with IFNγ which was used as positive control.

Phenotypes of lymphomononuclear cells after peptide stimulation

Surface markers in the cell populations after incubation in the presence of the active peptides were also studied. Table 5 shows the results obtained together with those corresponding to IL-2 or SpA. The percentage of CD3 and CD8 remained constant in the different samples. However, the proportion of cells expressing markers CD11b (NK, monocytes and macrophages) and CD14 (monocytes and macrophages) increased in the presence of SpA or active peptides. The percentage of these markers remained unaltered in populations incubated in the presence of inactive control

Table 5
Phenotypes of lymphomononuclear cells after peptide activation

Product tested	Cells expressing surface markers (%)					
	CD3	CD8	CD11b	CD14	CD16	CD56
Medium	59 ± 3	17 ± 2	14 ± 4	9 ± 1	11 ± 5	6 ± 2
Peptide No. 56	60 ± 1	17 ± 1	23 ± 5	17 ± 4	22 ± 7	18 ± 6
Peptide No. 13	58 ± 2	18 ± 2	24 ± 3	18 ± 3	22 ± 6	17 ± 5
Peptide No. 25	59 ± 3	17 ± 2	23 ± 4	18 ± 3	21 ± 5	18 ± 5
Peptide No. 81	58 ± 3	18 ± 2	21 ± 3	17 ± 4	21 ± 6	17 ± 5
Peptide No. 2	60 ± 2	17 ± 2	13 ± 4	9 ± 2	12 ± 4	6 ± 2
rIL-2	59 ± 3	19 ± 2	13 ± 2	6 ± 1	23 ± 4	25 ± 3
SpA	60 ± 3	18 ± 1	23 ± 4	16 ± 2	22 ± 5	20 ± 4

Lymphomononuclear cells were obtained from five different healthy donors. Results represent mean ± S.E. SpA concentration was 20 $\mu g/10^6$ cells. Peptide concentration was always 20 $\mu g/10^6$ cells. rIL-2 at a concentration of 500 $U/10^6$ cells was used as a reference control. Phenotypes were determined after 7 days of incubation.

Table 6
Release of Interleukin-1 and Tumour Necrosis Factor α following addition of cytotoxic peptides or control substances

Product tested	Factor release		
	IL-1 ($pg/10^6$ cells)	TNFα ($U/10^6$ cells)	TNFα + anti-TNFα
Medium	12	4	4
Peptide No. 56	68	89	16
Peptide No. 13	62	65	12
Peptide No. 25	39	63	10
Peptide No. 81	39	71	12
Peptide No. 2	5	7	6
LPS	150	–	–
PMA	–	540	17
IL-2	5	5	6
SpA	84	143	20

Lymphomononuclear cells were obtained from five different healthy donors. Results represent mean ± S.E.SpA concentration was 20 $\mu g/10^6$ cells. Peptide concentration was always 20 $\mu g/10^6$ cells; rIL-2, 500 $U/10^6$ cells; LPS 10 $\mu g/ml$; PMA (100 nM). Incubation time, 24 h.

peptides, or in the presence of IL-2. On the contrary, the expression of CD56 (NK and LAK) was higher in cells incubated in the presence of IL-2, than in the presence of SpA or active peptides. CD16 (neutrophils, monocytes and NK) was elevated in all cell populations with respect to the incubated controls.

Release of interleukin-1 and TNFα induced by synthetic peptides

In order to ascertain the possible stimulation of monocytes, IL-1 and TNFα were determined in the supernatants after 24 h of incubation. The results of Table 6 clearly show that both cytokines were released by cells incubated in the presence of either SpA or the active peptides. As expected, no increase was observed in the supernatants from cells incubated in the presence of IL-2. Lipopolysaccaride (LPS) and phorbol myristate acetate (PMA) were used as positive controls. The nature of TNFα released was also confirmed with anti-TNFα antibody. Preliminary results show also that isolated monocytes incubated in the presence of active peptides responded with the production of IL-1 and TNFα in a time-dependent fashion (data not shown).

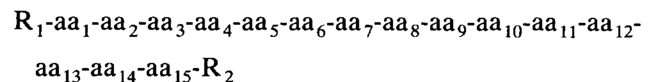
Cytotoxicity induced by active peptides on lymphomononuclear cells from cancer patients

The response to immunmodulating peptides was also studied in lymphomononuclear cells from cancer patients. Table 7 shows that peptides 13, 25 and 56, but not peptide 2, used as a control, induced an increase in cytotoxicity of these cells against K562 and Daudi cells. The values were lower than those elicited by IL-2. The changes of surface markers were similar to those observed with cells obtained from healthy donors (data not shown).

Cytotoxicity induced by active peptides on isolated monocytes

Isolated monocytes incubated for 5 days in the presence of SpA or active peptides exhibited a cytotoxicity against HT-29 cells higher than those incubated in the presence of an inactive control peptide (Table 8). The optimum effector-to-target cell ratio was 2:1.

In conclusion, the results clearly show that a number of synthetic peptides can induce cytotoxicity against cells of tumoral lines K562 and Daudi cells (Tables 1–4). The active peptides have a number of features which can be summarized in the following pattern:



$$R_1\text{-}aa_1\text{-}aa_2\text{-}aa_3\text{-}aa_4\text{-}aa_5\text{-}aa_6\text{-}aa_7\text{-}aa_8\text{-}aa_9\text{-}aa_{10}\text{-}aa_{11}\text{-}aa_{12}\text{-}aa_{13}\text{-}aa_{14}\text{-}aa_{15}\text{-}R_2$$

Table 7
Cytotoxicity induced by synthetic peptides on lymphomononuclear cells from cancer patients

Patients	Product tested						
	Medium	IL-2	SpA	Peptide			
				No. 13	No. 56	No. 25	No. 2
(a)							
A	12	58	42	21	26	19	12
B	7	46	56	49	44	52	12
C	32	60	68	46	50	56	29
D	6	88	56	39	41	48	12
(b)							
A	3	98	67	40	58	50	6
B	14	38	41	29	32	40	9
C	21	48	61	36	46	43	18
D	14	38	41	29	32	40	9

SpA concentration was 20 $\mu g/10^6$ cells. Peptide concentration was always 20 $\mu g/10^6$ cells. rIL-2 at a concentration of 500 $U/10^6$ cells was used as a reference control. Cytotoxicity was determined after 7 days of incubation. Effector cell/Target cell ratio was 50/1. The percentage of Chromium release of top (a) and bottom (b) panels were obtained against K562 and Daudi cells, respectively.
Patients: A, melanoma (stage III); B, non-Hodgkin lymphoma; C, breast carcinoma; D, renal cell carcinoma.

Table 8
Cytotoxicity induced by synthetic peptides on isolated monocytes against HT-29 cells

Product tested	Cytotoxic activity (% [^{3}H]thymidine release)
Medium	20±3
Peptide No. 56	60±4
Peptide No. 13	56±5
Peptide No. 25	58±6
Peptide No. 81	50±4
Inactive peptide No. 2	20±4
SpA	62±6
IFNγ	73±5

Monocytes were obtained from five different healthy donors. Results represent mean±S.E. SpA concentration was 20 μg/10^6 cells. Peptide concentration was always 20 μg/10^6 cells. IFNγ (100 U/10^6 cells) was used as reference control. Cytotoxicity was determined after 5 days of incubation. Effector-to-target cell ratio was 2:1.

where aa_6 must be Pro, aa_2 can be Val, Ile, Leu, Gly, Ala or Lys, and aa_{11} can be Glu or Asp.The activity of the peptides is maintained if $R_1 = H$ and $R_2 = OH$, or if R_1 or R_2 are longer peptide chains as the ones found in SpA.

The cytotoxicity seems to be mediated by NK, LAK and monocytes as suggested by the cell markers (Table 5); effect on K562 cells (NK dependent), Daudi (LAK and NK dependent) and HT-29 (monocyte dependent); and the release of IL-1 and TNFα (Table 6). Zembala et al. [25] have proposed that the endogenous production of TNFα is possibly involved in the enhancement of antigen presentation by monocytes in an autocrine fashion. The TNFα release induced by the active peptides discussed above could be an important step in the immunopotentiating effects observed.

These results allow also to hypothesize that the stimulation of the different cells involved in the response induced by the active peptides described could have been initiated through monocytes acting as APC.

Cytotoxicity against K562 and Daudi cells was also stimulated by the active peptides in lymphomononuclear cells from a number of cancer patients (Table 7). These results suggest the possibility of using these peptides as a potential cancer therapy.

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