



Anti-tumor activities of peptides corresponding to conserved complementary determining regions from different immunoglobulins



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ABSTRACT

Short synthetic peptides corresponding to sequences of complementarity-determining regions (CDRs) from different immunoglobulin families have been shown to induce antimicrobial, antiviral and anti-tumor activities regardless of the specificity of the original monoclonal antibody (mAb). Presently, we studied the *in vitro* and *in vivo* antitumor activity of synthetic peptides derived from conserved CDR sequences of different immunoglobulins against human tumor cell lines and murine B16F10-Nex2 melanoma aiming at the discovery of candidate molecules for cancer therapy. Four light- and heavy-chain CDR peptide sequences from different antibodies (C36-L1, HA9-H2, 1-H2 and Mg16-H2) showed cytotoxic activity against murine melanoma and a panel of human tumor cell lineages *in vitro*. Importantly, they also exerted anti-metastatic activity using a syngeneic melanoma model in mice. Other peptides (D07-H3, MN20v1, MS2-H3) were also protective against metastatic melanoma, without showing significant cytotoxicity against tumor cells *in vitro*. In this case, we suggest that these peptides may act as immune adjuvants *in vivo*. As observed, peptides induced nitric oxide production in bone-marrow macrophages showing that innate immune cells can also be modulated by these CDR peptides. The present screening supports the search in immunoglobulins of rather frequent CDR sequences that are endowed with specific antitumor properties and may be candidates to be developed as anti-cancer drugs.

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Introduction

Chemotherapy is a major approach to treat various cancers. Side effects, however, are commonly reported because of the inability to deliver the appropriate amount of drug specifically to tumor cells without affecting normal cells. Drug resistance, clearance and biodistribution are also problems to be faced in the various treatment protocols [7]. Aiming at specifically targeting tumor cells and endothelial cells of neoangiogenesis, or to stimulate the immune system, proteins, monoclonal antibodies and peptides have been used [26]. Due to size restrictions, antibodies and large protein ligands may have poor delivery to tumors and exhibit toxicity to the

liver and bone marrow. In contrast, peptides have the advantage of small size, good tumor penetration and biocompatibility.

Apart from the immune peptides derived from tumor cell protein antigens that are mostly known as T CD8⁺ epitopes, anti-cancer peptides can act directly through inhibition of angiogenesis, induction of apoptosis or by inhibiting protein–protein interactions, signal transduction pathways, enzymes and gene expression [7,10,20,26,27]. Some of them penetrate tumor cells and remodel actin cytoskeleton with inhibitory effects on cell migration [8] or severely affect actin dynamics leading tumor cells to apoptosis [2].

Short-sized peptides can act directly on tumor cells without affecting normal cells, thus evolving as an alternate strategy to conventional chemotherapy [26]. Peptides can also act as carriers of cytotoxic agents and radioisotopes conveying them to the tumor microenvironment. Those derived from native proteins have been described to present antimicrobial activities similar to peptides of innate immunity (defensins, cathelicidins, histatins) [6]. More than 30 peptides and their oncolytic properties against solid tumors have been recently reviewed [13].

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In a previous work, Polonelli et al. [24] showed that synthetic CDR peptides can exhibit different antimicrobial, antiviral and antitumor activities *in vitro*, *in vivo* and/or *ex vivo*, in a way that was compared to early molecules of innate immunity [17]. This contribution extended previous results showing the remarkable therapeutic activity of an engineered synthetic peptide included in the variable region of a recombinant yeast killer toxin-like anti-idiotypic antibody against experimental mucosal and systemic candidiasis [23]. Both articles opened a new field of interest focusing on the bioactivity of internal sequences of immunoglobulins [18]. As a prototype of the CDR bioactive peptides, C7H2 derived from mAb C7 V_H CDR 2, showed significant antitumor activity *in vitro* and *in vivo* [24] notwithstanding the original specificity of the mAb, directed to a *Candida albicans* cell wall mannoprotein. C7H2 induces apoptosis in melanoma cells, by interacting with, and altering β -actin dynamics associated with abundant generation of superoxide anions [2]. Additional evidence of IgM/IgG-CDR-derived synthetic peptides with antitumor activities, including the description of microantibodies, has also been obtained [9]. The prototype, a 17-amino acid microantibody, derived from a mouse mAb was able to inactivate HIV *in vitro* [14].

Recent evidence on immunomodulatory activities of Ig-CDR peptides has also been obtained [11,19]. Cytotoxic effects have also been described for constant regions of immunoglobulins [12,22]. As a proof of concept of the bioactive potential of Ab CDR peptide sequences, we extended these studies and report on the differential antitumor activities of synthetic peptides with sequences identical to CDRs of the light (L₁ and L₃) and heavy (H₂ and H₃) chains of different immunoglobulin families. The CDRs chosen have more often been implicated in bioactivity than L₂ and H₁. We show here the biological activity of different CDR peptides as promising molecules displaying relevant *in vitro* and *in vivo* antitumor activities against malignant melanoma.

Experimental

CDR peptide design and synthesis

The CDR amino acid sequences were obtained according to the rules of Kabat [15] and Chothia [1,4] beyond the AbM method, or “definition of AbM” created from Accelrys software (Oxford Molecular’s AbM or antibody modeling software), as described. The protein BLAST program of NCBI-NLM was used for choosing the CDR sequences with more than 80% homology within a family of immunoglobulins. Priority was given to CDRs derived from variable light chains L₁ and L₃ and heavy chains H₂ and H₃. Table 1 shows the different sequences of peptides sorted out for the present investigation.

Tumor cell lines and cell culture

The murine B16F10 cell line of melanoma cells was subcloned at the Experimental Oncology Unit, Federal University of São Paulo, UNIFESP and the cell line B16F10-Nex2 was obtained and deposited at Banco de Células Rio de Janeiro (BCRJ 0342). Cell lineages of human melanoma (A2058), human colon carcinoma (HCT), human breast cancer (MCF-7 and SKBR-3), mouse fibroblasts (3T3), murine melanocytes (melan-A) and human foreskin fibroblast (HF) were provided by Ludwig Institute for Cancer Research, São Paulo, Brazil and Dr. Luiz F. Lima Reis (Hospital Sirio-Libanez, São Paulo). Cells were cultured at 37 °C, under humid atmosphere and 5% CO₂, in RPMI-1640 medium with 10 mM N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES), 24 mM sodium bicarbonate, 40 mg/L gentamicin, pH 7.2 and 10% fetal calf serum (FCS). U87-MG cell line

Table 1

Amino acid sequences of 17 CDR peptides with their respective access codes from NCBI BLAST.

Peptide	Amino acid sequence	Access codes
LA3-L1	RASQGISSWLA	BAC80166.1
B9-L1	RASQSISSYLN	AEA48887.1
C36-L1	KSSQSVFYSSNNKNYLA	CAB44483.1
MS1-L1	TLSSGHSSYAIA	AAS21063.1
MN20-L3	QQYSGYPY	2BRR.L
V330-H2	VISYDGSYKYADSVKG	BAI51287.1
CM04A05-H2	VISGSSGRTHYADSVKG	CAL06666.1
D2-H2	GIPIFGAANYAQ	ABV70971.1
CE48-H2	INSGGGGTYADSVKG	CAL04002.1
Mg16-H2	EIYYSSTNYPNPSLKS	ABI35660.1
1-H2	SLYYSSTTFYNPSLKS	CAD44679.1
HA9-H2	YIYYSSTNYPNPSLKS	AAO86898.1
D07-H3	DLRNHVFWSGYSTSFYD	ABE97346.1
A7-H3	DRGRGLISYYYGMVDV	AAW29179.1
F1-H3	ASYGSRGWYFDV	CAA80000.1
F6-H3	DQGGDDYGDYYYGYGMDV	ABP98185.1
MS2-H3	DTVMATPYFDY	AAM88150.1

(glioblastoma) was provided by Dr. Osvaldo K. Okamoto, University of São Paulo and was maintained in DMEM medium supplemented as for the RPMI-1640 medium.

Cell viability assay

Tumor cells (10⁴) were seeded in 96-well plates and incubated with all peptides at different concentrations ranging from 0 to 1 mM. Viable cells were quantified using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma–Aldrich, St. Louis, MO) assay. After 24 h of incubation with peptides, 10 μ l of MTT solution (5 mg/ml) in 16 phosphate-buffered saline (PBS) was directly added to the cells, followed by incubation for 3 h at 37 °C. Absorbance was measured at 570 nm with an automated spectrophotometric plate reader (SpectraMax-M2, Molecular Devices Software Pro 5.4, Sunnyvale, CA). Cell viability was expressed as percent values in comparison with untreated control cells. The experiments were performed in triplicate. Alternatively, viable cells were quantified using the Trypan Blue exclusion assay.

Murine bone marrow cells and macrophage differentiation

Fresh bone marrow cells were used to generate macrophages using L929-cell supernatant conditioned medium (LCCM) as a source of granulocyte/macrophage-colony stimulating factor (GM-CSF). Cells were resuspended in 10 ml bone marrow differentiation medium, which consists of RPMI-1640 (Gibco, Grand Island, NY) supplemented with 20% fetal bovine serum (Gibco, Grand Island, NY), 30% LCCM, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine. Cells were seeded in non-tissue culture treated Optilux Petri dishes (BD Biosciences, Franklin Lakes, NJ) and incubated at 37 °C in 5% CO₂ atmosphere. After 4 days, 10 ml of fresh medium was added per plate and incubated for additional 3 days. To obtain macrophages, the supernatants were discarded and the adherent cells were washed with 15 ml of sterile PBS. Macrophages were detached gently using a cell scraper and PBS. The cells were centrifuged at 200 \times g for 5 min and resuspended in 10 ml of RPMI 1640 (Gibco, Grand Island, NY). Cells were counted and cultured in tissue culture plates for 12 h.

Macrophage NO production

Nitric oxide (NO) production was assessed by measuring the accumulation of nitrite in the culture medium using the Griess reaction. 5 \times 10⁵ Bone marrow macrophages were seeded in 24-well

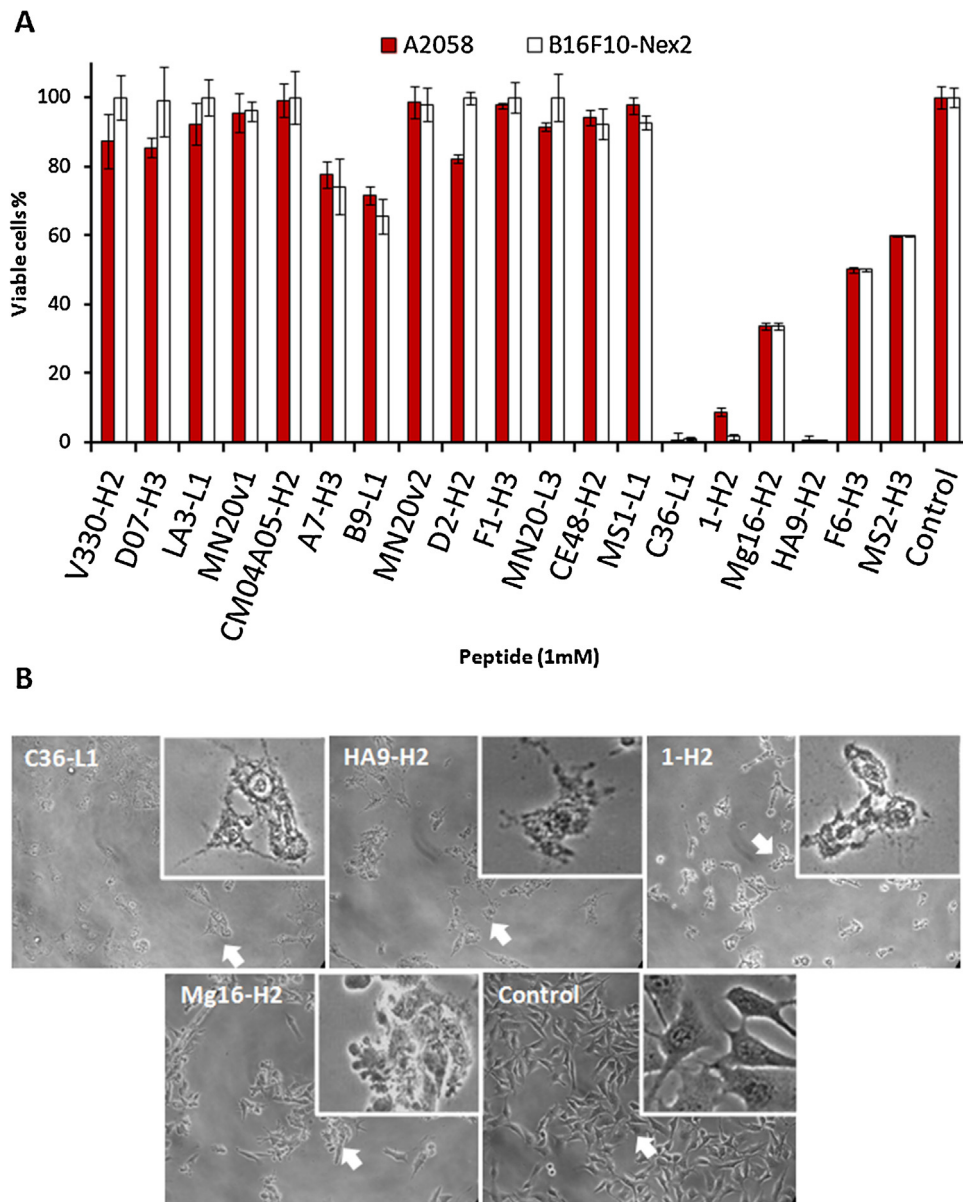


Fig. 1. (A) Cytotoxicity of 17 CDR derived and 2 homologous *Drosophila* peptides in murine melanoma B16F10-Nex2 and human melanoma A2058 cells. Peptides were incubated with tumor cells at 1 mM for 24 h and viability was accessed by MTT method. (B) Tumor cells morphology after incubation with peptides C36-L1, HA9-H2, 1-H2 and Mg16-H2. All experiments were made in triplicate.

plates and incubated at 37 °C, 5% CO₂ for 6 h. Adherent macrophages were stimulated with LPS (200 ng/ml), IFN- γ (100 U/ml) or a combination of the two in the presence or not of each peptide for 72 h. Supernatants were harvested and mixed with equal volumes of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) and incubated at room temperature for 10 min. NO was quantified by absorption in a microplate reader at 550 nm (Spectramax m2e, Molecular Devices), and converted based on a nitrite standard curve [5]. Untreated cells and cells treated with IFN- γ or LPS or both without adding the peptides were the controls used.

Metastatic melanoma assay

Male, 7–8-week-old C57Bl/6 mice were injected endovenously with 5×10^5 syngeneic B16F10-Nex2 viable cells in 0.1 ml RPMI per animal. For antitumor protection experiments, two groups of mice were challenged with tumor cells and treated on the next

day with intraperitoneal (i.p.) doses of 300 μ g peptide in 100 μ l of PBS during 5 consecutive days. After 12–15 days, lungs were collected and inspected for metastatic colonization and the black metastatic nodules counted. Animals challenged e.v. with tumor cells and treated with PBS alone without peptides were the controls used.

Results

CDR peptides studied in the present work were sorted out from monoclonal antibodies reported in the literature to specifically recognize antigenic epitopes on eukaryotic and prokaryotic cells, and viruses. Table 1 shows the different amino acid sequences of CDR peptides investigated in the present work. Aiming at an initial screen for bioactivity using macrophages and tumor cells as targets the various peptides were tested for cytotoxicity effects, NO production and protective activities using a syngeneic murine metastatic melanoma model.

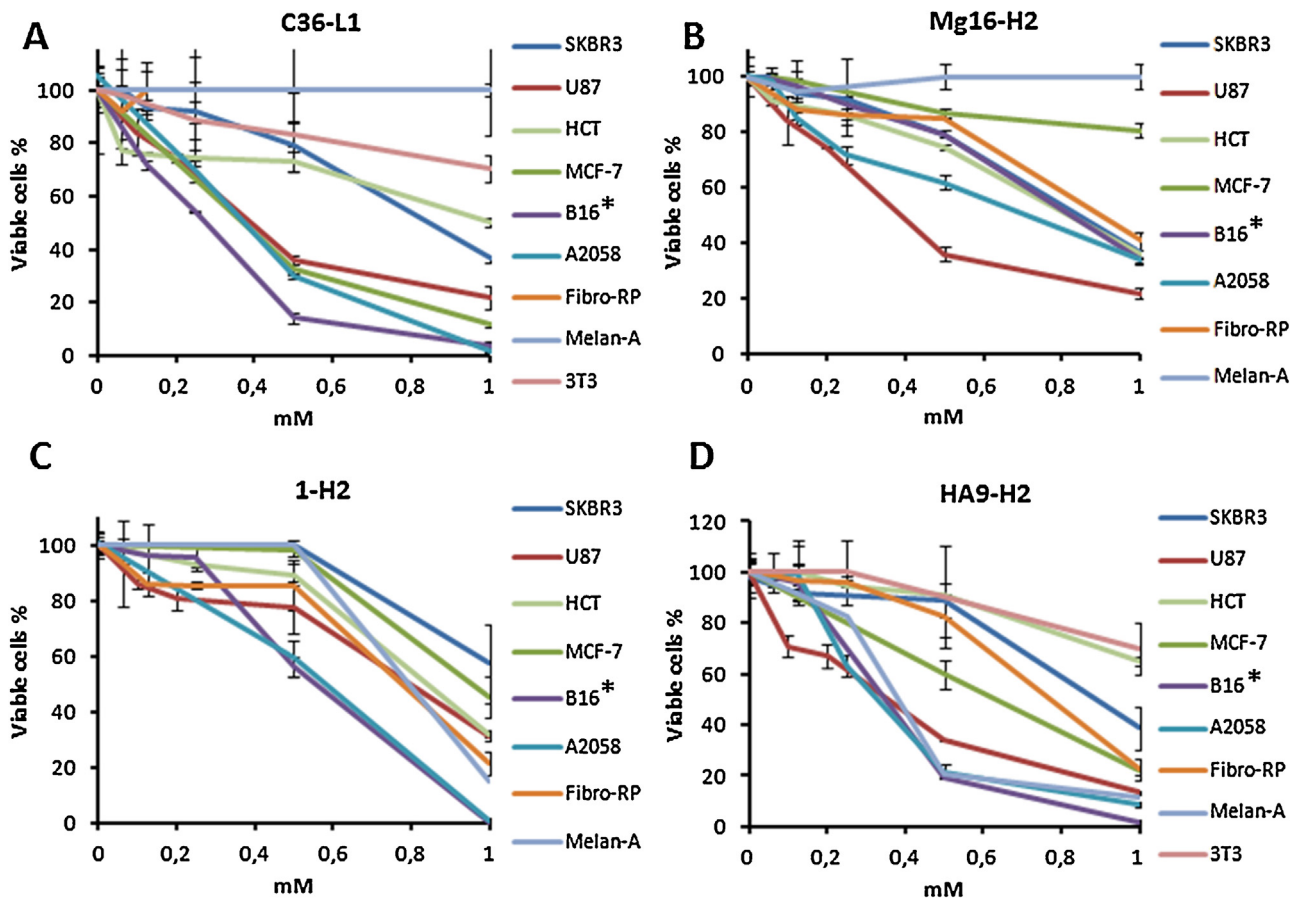


Fig. 2. Dose dependent response to (A) C36-L1, (B) Mg16-H2, (C) 1-H2 and (D) HA9-H2 CDR peptides in different tumor cell lines and non-tumorigenic cells. 10^4 Cells were incubated with different concentrations of each peptide ranging from 0 to 1 mM. *B16 = B16F10-Nex2. Results are means of three independent experiments.

In vitro cytotoxicity of CDR peptides

The cytotoxicity of all CDR peptides was evaluated on B16F10-Nex2 murine melanoma and A2058 human melanoma cells at 1 mM for 24 h. We observed that peptides C36-L1, HA9-H2, 1-H2 and Mg16-H2 were the most cytotoxic and significantly reduced tumor cells viability as shown in Fig. 1A. In Fig. 1B, morphological alterations in B16F10-Nex2 cells incubated with peptides C36-L1, HA9-H2, 1-H2 and Mg16-H2 are shown. White arrows and inserts reveal cytoplasm shrinkage in melanoma cells treated with peptides C36-L1, HA9-H2 and 1-H2, and partial fragmentation into discrete bodies, which are frequently referred to as 'apoptotic bodies' [16], with peptide Mg16-H2. Peptides F6-H3, MS2-H3, B9-L1 and A7-H3 also showed a significant activity *in vitro*, partially affecting tumor cells viability. We also tested two *Drosophila* peptides analogous to MN20-L3 CDR, the MN20v1 (QQYSGYPY-NH₂) and MN20v2 (QQYSGYPY-NH₂). Both peptides, however, were not active against tumor cells *in vitro*.

The growth inhibition of the most active peptides *in vitro*, C36-L1, HA9-H2, 1-H2 and Mg16-H2, was evaluated on different tumor and non-tumorigenic cell lineages as shown in Fig. 2 and the IC₅₀ values determined (Table 2). Peptide C36-L1 was the most active peptide found in this screening showing low IC₅₀ values (0.23–0.5 mM) against murine melanoma B16F10-Nex2, human melanoma A2058, human glioblastoma U87 and human breast cancer MCF-7. This peptide was not inhibitory against non-tumorigenic cells including melan A, as indicated in Fig. 2A and Table 2. In contrast, peptide HA9-H2 significantly inhibited non-tumorigenic cells, except for NIH-3T3 fibroblasts. Peptides Mg16-H2 and 1-H2

were less active against all tumor cells as shown in Fig. 2B and C and Table 2.

In vivo anti-metastatic assay

The *in vivo* antitumor activity of all peptides was investigated in a syngeneic murine metastatic model, with B16F10-Nex2 tumor cells and C57BL/6H-2^b mice. We observed that peptides C36-L1, HA9-H2, 1-H2, Mg16-H2, D07-H3, MN20v1 and MS2-H3 induced significant protection against metastatic melanoma reducing the number of lung metastatic nodules (Fig. 3). No protection was observed with the other CDR peptides (LA3-L1, B9-L1, MS1-L1, MN20-L3, V330-H2, CM04A05-H2, D2-H2, CE48-H2, A7-H3, F1-H3, F6-H3) and the *Drosophila* MN20v2 analog. Responses were

Table 2

IC₅₀ values obtained for C36-L1, 1-H2, HA9-H2 and Mg16-H2 CDR peptide activities against different tumor cell and non-tumorigenic cell lineages.

Cell lineage	Peptide-IC ₅₀ (mM)			
	C36-L1	1-H2	HA9-H2	Mg16-H2
B16F10-Nex2	0.235	0.56	0.45	0.82
A2058	0.35	0.53	0.25	0.70
U87	0.50	0.76	0.36	0.95
SKBr-3	0.84	>1	0.9	>1
HCT	0.95	0.89	0.82	>1
MCF-7	0.3	0.98	0.63	>1
Melan-A	>1	0.85	0.32	>1
Fibro-RP	>1	0.82	0.78	0.94
NIH-3T3	>1	0.92	>1	>1

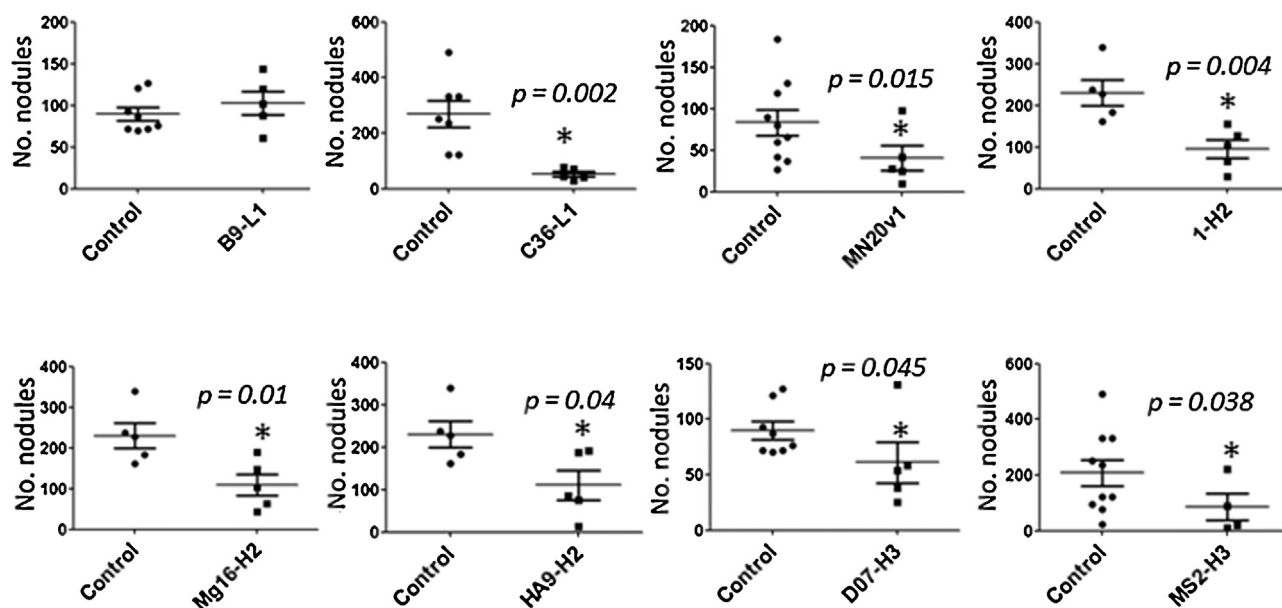


Fig. 3. Antimetastatic activity of CDR peptides. 5×10^5 B16F10-Nex2 cells were injected endovenously in C57Bl/6 mice and doses of 300 $\mu\text{g/day}$ of each peptide were administered during five consecutive days. The animals were euthanized on day 15, their lungs were surgically excised and the number of metastatic nodules quantified under a Stereo Microscope (Nikon). At least three experiments were made for each peptide.

similar to that observed for B9-L1 peptide (Fig. 3). In addition, no toxic effects, loss of weight, or alterations in animal behavior were observed during the treatment with all peptides.

In vitro macrophage activation assay

Peptides C36-L1 and 1-H2, which were both active against B16F10-Nex2 cells *in vitro* and *in vivo*, also induced significant production of NO in IFN- γ -induced macrophages (Fig. 4). D07-H3 elicited high NO production in macrophages and also induced significant antitumor protection *in vivo*. Peptide MN20v1, which elicited significant antitumor protection *in vivo* and NO production *in vitro*, was not directly cytotoxic against B16F10-Nex2 cells (Fig. 1), suggesting participation of the immune system. MS2-H3, which was protective *in vivo*, induced significant NO production in IFN- γ and LPS activated macrophages, and was moderately

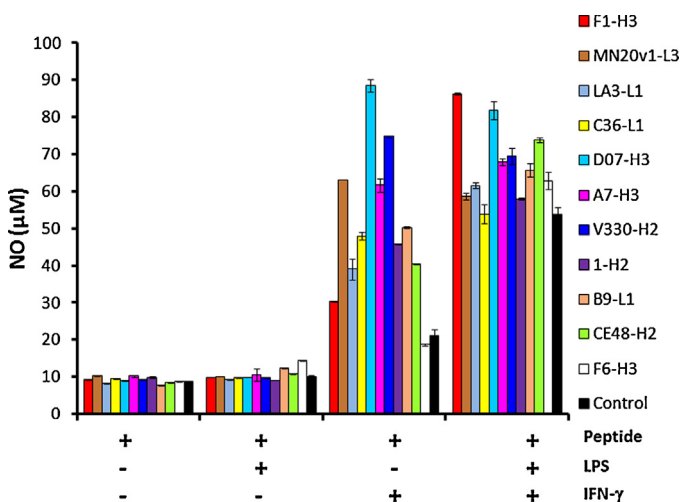


Fig. 4. Macrophage NO production induced by CDR peptides. Peptides at 100 μM , were incubated for 72 h with macrophages in the presence or absence of LPS (200 $\mu\text{g/ml}$) and IFN- γ (100 U/ml). NO production was assayed using 100 μl of Griess reagent. Controls (black bars) were run without the peptides, for each group. Two experiments were made with four determinations for each peptide.

active against B16F10-Nex2 cells *in vitro*. F1-H3 also induced NO production in IFN- γ activated macrophages but was poorly protective *in vivo* (Fig. 3). NO production by IFN- γ and LPS treated macrophages treated with the other CDRs (LA3-L1, B9-L1, MS1-L1, MN20-L3, V330-H2, CM04A05-H2, D2-H2, CE48-H2, Mg16-H2, HA9-H2, A7-H3, F6-H3), was similar or lower than that with MS1-L1 peptide.

Discussion

Following the pivotal work by Polonelli et al. [24] further research on the antitumor activity of CDRs [2,9,19] showed a frequency of 1–2 CDRs per monoclonal antibody exerting anti-cancer properties *in vitro*, *in vivo* or both. Of 6 CDRs, the most frequently active were L1, H2 and H3. In the present work we confirmed this preference testing 17 CDRs and 2 analogous *Drosophila* peptides.

Screening of active CDRs used the corresponding synthetic peptides amidated at the C terminal and included a short number of *in vitro* and *in vivo* tests. Most tests used B16F10-Nex2 murine melanoma cells, and a few were run with a panel of human cancer lineages and also with non-tumorigenic cells.

Since several CDR peptides induced apoptosis in tumor cells [24], the morphological characteristics of cells incubated with lethal concentrations of peptides could indicate programmed cell death (PCD), including rounding-up of cells, cytoplasm shrinkage, chromatin condensation, nuclear fragmentation and plasma membrane blebbing [16]. PCD may refer to apoptosis but also to autophagy and even programmed necrosis when mediated by an intracellular program deciding the fate of cells of malignant neoplasms [21].

The therapeutic activity of anticancer agents is limited by their toxicity exerted in proliferating cells, including normal cells [3]. Novel cytotoxic agents preferentially targeting cancer cells are thus a constant goal. In the present study, peptide C36-L1 emerged as a promising candidate for targeted cancer therapy.

The antitumor activity *in vivo* observed after systemic treatment with peptides could be related to a direct activity on the tumor, evidenced by the cytotoxic potential of peptides C36-L1, HA9-H2 and 1-H2, but also as an effective participation of the immunological system since it has been reported that certain peptides

are not protective in immune deficient animals [19]. In fact, the intraperitoneal injections in consecutive days used in our protocols with unprotected peptides, probably would not preclude proteolytic degradation and renal clearance, unless peptides are efficiently taken up by dendritic cells (DCs). Tumor antigen-primed DCs treated with CDR peptides conferred anti-tumor protection similar to the free peptide [19]. Therefore in those cases in which peptides were protective *in vivo* but poorly cytotoxic *in vitro* most probably an immune response was likely involved. Peptides D07-H3, MN20v1 and MS2-H3, that were clearly protective *in vivo*, had poor or no significant activity against murine melanoma B16F10-Nex2 cells *in vitro*, suggesting effects as adjuvants in protective immune responses.

Effector cells of innate immunity include granulocytes, natural killer cells and macrophages, all of them playing an important role in the antitumor immunity. In addition to the already described effects of CDR peptides, an immunomodulatory activity has been attributed to the CDR H3 from an anti-blood group A murine mAb. The peptide acted on macrophages, stimulating proinflammatory cytokine production, activating the PI3K–Akt pathway and promoting TLR-4 expression [11]. We observed that some CDR peptides that showed antitumor protection *in vivo* also induced significant NO production in IFN- γ -induced macrophages, providing another evidence that these peptides may act in the activation of an immune protective response. Recently, we showed (Figueiredo CR, unpublished results) that C36-L1-treated DCs were protective in the B16F10-Nex2 metastatic model, thus furthering the notion that CDR peptides may act as potent adjuvants for anti-tumor therapy.

Although a protective immune response against growing tumors may occur spontaneously, it usually fails to cause tumor regression. The use of immune adjuvants might be effective against early-stage developing tumors. It is questionable whether *ex vivo*-prepared DC vaccines are superior to *in situ*-administered adjuvant-guided vaccines [25]. Peptides have been described to protect against subcutaneous B16F10-Nex2 melanoma even when tumors reached 100–200 mm³ [19,20].

In conclusion, the evidence that Igs are a source of anti-tumor internal peptides mainly represented by the hypervariable CDRs is supported in the present work. CDR peptides from different families of immunoglobulins display a number of biological activities, such as direct activity on tumor cells and acting as immune adjuvants stimulating antitumor responses *in vivo*. Peptides can be synthesized in high amounts and purity and be kept lyophilized without loss of activity. They are inexpensive, naturally biodegradable and well tolerated. Some of the peptide sequences described, with anti-tumor activities *in vitro* and *in vivo* in a mouse syngeneic system, can be further studied aiming at a molecular therapy of malignant melanoma and other tumors.

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References

- [1] Al-Lazikani B, Lesk AM, Chothia C. Standard conformations for the canonical structures of immunoglobulins. *J Mol Biol* 1997;273:927–48.
- [2] Arruda DC, Santos LC, Melo FM, Pereira FV, Figueiredo CR, Matsuo AL, et al. Beta-actin-binding complementarity-determining region 2 of variable heavy chain from monoclonal antibody C7 induces apoptosis in several human tumor cells and is protective against metastatic melanoma. *J Biol Chem* 2012;287:14912–22.
- [3] Chari RV. Targeted cancer therapy: conferring specificity to cytotoxic drugs. *Accounts Chem Res* 2008;41:98–107.
- [4] Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. *J Mol Biol* 1987;196:901–17.
- [5] Clarke JH, Cha JY, Walsh MD, Gamboni-Robertson F, Banerjee A, Reznikov LL, et al. Melanoma inhibits macrophage activation by suppressing toll-like receptor 4 signaling. *J Am Coll Surg* 2005;201:418–25.
- [6] De Smet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett* 2005;27:1337–47.
- [7] Deepak KDJ, Shrivastava V, Kakde R, Patil AT. Cancer therapeutics – opportunities, challenges and advances in drug delivery. *J Appl Pharm Sci* 2011; 01:10.
- [8] Delaroché D, Cantrelle FX, Subra F, Van Heijenoort C, Guittet E, Jiao CY, et al. Cell-penetrating peptides with intracellular actin-remodeling activity in malignant fibroblasts. *J Biol Chem* 2010;285:7712–21.
- [9] Dobroff AS, Rodrigues EG, Juliano MA, Friaca DM, Nakayasu ES, Almeida IC, et al. Differential antitumor effects of IgG and IgM Monoclonal antibodies and their synthetic complementarity-determining regions directed to new targets of B16F10-Nex2 melanoma cells. *Transl Oncol* 2010;3: 204–17.
- [10] Eldar-Finkelman H, Eisenstein M. Peptide inhibitors targeting protein kinases. *Curr Pharm Des* 2009;15:2463–70.
- [11] Gabrielli EPE, Cenci E, Ortelli F, Magliani W, Ciociola T, Bistoni F, et al. Antibody complementarity-determining regions (CDRs): a bridge between adaptive and innate immunity. *PLoS One* 2009;4.
- [12] Gabrielli E, Pericolini E, Cenci E, Ortelli F, Magliani W, Ciociola T, et al. Antibody constant region peptides can display immunomodulatory activity through activation of the Dectin-1 signalling pathway. *PLoS One* 2012;7:e43972.
- [13] Gaspar D, Veiga AS, Castanho MA. From antimicrobial to anticancer peptides. A review. *Front Microbiol* 2013;4:294.
- [14] Heap CJ, Wang Y, Pinheiro TJ, Reading SA, Jennings KR, Dimmock NJ. Analysis of a 17-amino acid residue, virus-neutralizing microantibody. *J Gen Virol* 2005;86:1791–800.
- [15] Kabat EA. Sequences of proteins of immunological interest. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1991.
- [16] Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, et al. Classification of cell death: recommendations of the Nomenclature Committee on cell death 2009. *Cell Death Differ* 2009;16:3–11.
- [17] Litman GW, Cannon JP, Dishaw LJ. Reconstructing immune phylogeny: new perspectives. *Nat Rev Immunol* 2005;5:866–79.
- [18] Magliani W, Conti S, Cunha RL, Travassos LR, Polonelli L. Antibodies as crypts of anti-infective and antitumor peptides. *Curr Med Chem* 2009;16: 2305–23.
- [19] Massaoka MHMA, Scutti JAB, Arruda DC, Rabaça A, Figueiredo CR, Farias CF, et al. Melanoma: perspectives of a vaccine based on peptides. In: *Molecular vaccine*. Wien: Springer-Verlag; 2013.
- [20] Massaoka MH, Matsuo AL, Figueiredo CR, Girola N, Faria CF, Azevedo RA, et al. A novel cell-penetrating peptide derived from WT1 enhances p53 activity, induces cell senescence and displays antitumor activity in xeno- and syngeneic systems. *FEBS Open Bio* 2014;4:153–61.
- [21] Ouyang L, Shi Z, Zhao S, Wang FT, Zhou TT, Liu B, et al. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif* 2012;45:487–98.
- [22] Polonelli L, Ciociola T, Magliani W, Zanello PP, D'Adda T, Galati S, et al. Peptides of the constant region of antibodies display fungicidal activity. *PLoS One* 2012;7:e34105.
- [23] Polonelli L, Magliani W, Conti S, Bracci L, Lozzi L, Neri P, et al. Therapeutic activity of an engineered synthetic killer anti-idiotypic antibody fragment against experimental mucosal and systemic candidiasis. *Infect Immun* 2003;71:6205–12.
- [24] Polonelli L, Ponton J, Elguezal N, Moragues MD, Casoli C, Pilotti E, et al. Antibody complementarity-determining regions (CDRs) can display differential antimicrobial, antiviral and antitumor activities. *PLoS One* 2008;3: e2371.
- [25] Schijns VTE, Michalek J, Stathopoulos A, Dobrovolskienė NT, Strioga MM. Immune adjuvants as critical guides directing immunity triggered by therapeutic cancer vaccines. *Cytotherapy* 2014;16:13.
- [26] Thundimadathil J. Cancer treatment using peptides: current therapies and future prospects. *J Amino Acids* 2012;2012:967347.
- [27] Tonelli R, Purgato S, Camerin C, Fronza R, Bologna F, Alboresi S, et al. Anti-gene peptide nucleic acid specifically inhibits MYCN expression in human neuroblastoma cells leading to cell growth inhibition and apoptosis. *Mol Cancer Ther* 2005;4:779–86.