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Vaccine 22 (2004) 714–723
Vaccine
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Influenza virosomes enhance class I restricted CTL induction through CD4+ T cell activation

Reto Schumacher[a], Michel Adamina[a], Rinaldo Zurbriggen[b], Martin Bolli[a], Elisabetta Padovan[a], Paul Zajac[a], Michael Heberer[a], Giulio C. Spagnoli[a,c,*]

[a] *Departments of Surgery and Research, University of Basel, Basel, Switzerland*
[b] *Pevion Biotech, Bern, Switzerland*
[c] *ZLF, Lab 401, Hebelstrasse 20, CH-4031 Basel, Switzerland*

Received 28 March 2003; received in revised form 31 July 2003; accepted 20 August 2003

Abstract

Immunopotentiating reconstituted influenza virosomes (IRIV) are one of the few adjuvants currently licensed for human use. While their adjuvant capacity in the induction of humoral responses is clearly documented, few data exist on their effects on T cell immune response. Here we addressed IRIV adjuvance in the induction of HLA class I restricted cytotoxic T lymphocytes (CTL) in vitro.

Lymphocyte stimulation with $IM_{58–66}$ and IRIV resulted in marked expansion of specific CTL as compared to cultures performed in the presence of either antigen alone or antigen and control liposomes (L). Studies addressing underlying adjuvant mechanisms demonstrated that IRIV activated CD4/CD45RO+ T cells, induced a cytokine profile consistent with T helper 1 (Th1) stimulation and increased the percentage of CD4+ T cells expressing CXCR3. Furthermore, supernatants from IRIV stimulated PBMC cultures promoted dendritic cell maturation. Most importantly, IRIV mediated CTL adjuvance required the presence of live CD4+ T cells. Powerful adjuvant effects of IRIV were also observed in the induction of CTL specific for the melanoma associated Melan-A/MART-$1_{27–35}$, HLA-A0201 restricted epitope.

Taken together these findings indicate that IRIV are endowed with a high adjuvant capacity for HLA class I restricted CTL induction, largely attributable to their ability to antigenically stimulate CD4+ T cells.

Keywords: Virosomes; CTL; Adjuvance; T-helper 1

1. Introduction

Immunogenic preparations suitable for preventive and therapeutic vaccination purposes usually include specific antigens and adjuvants. The latter are administered to enhance immune responses although their mechanisms of action at the molecular level are frequently only poorly elucidated. Due to potentially severe side effects, only a limited number of adjuvants are currently licensed for use in humans [1].

A large majority of vaccine preparations induces humoral immune responses [1–3].

However, prevention or treatment of infectious diseases and active specific cancer immunotherapy frequently require the induction of powerful cellular immune responses. In particular, the development of immunization procedures capable of boosting class I restricted cytotoxic T lymphocytes (CTL) responses is urgently required. Remarkably, most adjuvants licensed for human use are ineffective in promoting CTL induction [3,4].

Immunopotentiating reconstituted influenza virosomes (IRIV) are liposomal preparations inclusive of influenza virus A/Singapore strain derived hemagglutinin [5,6]. After cell entry through receptor mediated endocytosis, they have the capacity to fuse with the endosomal membrane and to deliver encapsulated antigens into the cytosol [5–7].

IRIV have been shown to induce CTL responses specific for encapsulated peptides [7,8] “in vitro” and “in vivo”. These effects were largely attributed to antigen protection from enzymatic digestion and to cytosolic antigen delivery, features also shared by other liposomal preparations [7–11].

We have addressed adjuvant effects of IRIV independent from antigen delivery and preservation of antigen integrity. Here we show that IRIV are powerful adjuvants in the induction of class I restricted CTL responses. This capacity

* Corresponding author. Tel.: +41-61-265-2376; fax: +41-61-265-3990.
E-mail address: gspagnoli@uhbs.ch (G.C. Spagnoli).

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doi:10.1016/j.vaccine.2003.08.019

mainly relies on the stimulation of CD4+ T cell reactivity specific for viral proteins.

2. Materials and methods

2.1. Preparation of IRIVs

Egg phosphatidylcholine (PC, 32 mg), (Lipoid GmbH, Ludwigshafen, Germany) and phosphatidylethanolamine (PE, 8 mg), (R. Berchtold, Biochemisches Labor, Bern, Switzerland) were dissolved in 2.66 ml of PBS containing 100 mM octaethyleneglycol (OEG) (Fluka Chemicals, Switzerland), (PBS-OEG). The influenza A/Singapore hemagglutinin was purified as described previously [12]. A solution containing 2 mg hemagglutinin was centrifuged for 30 min at 100,000 × *g* and the pellet was dissolved in 1.33 ml of PBS-OEG. The phospholipids and the hemagglutinin-solution were mixed and sonicated for 1 min. This mixture was then centrifuged for 1 h at 100,000 × *g* and the supernatant sterile filtered (0.22 μ). Virosomes were then formed by detergent removal using SM Bio-Beads (Bio Rad, Hercules, PA). Final influenza hemagglutinin content was of 50 μg/ml, as determined by single radial diffusion [13]. Control liposomes (L) were similarly produced, in the absence of influenza virus components.

2.2. Cell cultures

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood of healthy donors or from umbilical cord blood samples by Ficoll gradient centrifugation. When indicated, specific PBMC subpopulations were purified by magnetic cell separation (Miltenyi Biotech, Bergisch Gladbach, Germany) according to producers' protocols. Cells were then cultured at 37 °C in RPMI 1640 supplemented with Kanamycin (100 μg/ml), Hepes (10 mM), sodium pyruvate (1 mM) Glutamax (1 mM) and non-essential amino acids (all from GIBCO Paisley, Scotland, thereafter referred to as complete medium) and 5% human serum (Blutspendezentrum Kantonsspital Basel Switzerland). Immature dendritic cells (iDC) were generated from CD14+ PBMC upon culture for 5–7 days in complete medium supplemented with FCS 10% (GIBCO), β-mercaptoethanol 0.004%, IL-4 (1000 U/ml, courtesy of Dr. Lanzavecchia, Bellinzona, Switzerland) and GM-CSF (50 ng/ml, Novartis, Basel, Switzerland).

2.3. Proliferation assays

Cells were cultured in the presence of IRIV, L, or in the absence of any stimuli in 96-well flat bottom tissue culture plates (Becton Dickinson, Le Pont de Claix, France) at 2×10^5 cells per well in triplicates for each condition. On day five, cultures were pulsed with [^{3}H]thymidine (Amersham, Little Chalfont, UK) (1 μCi per well) for 18 h and then harvested. Tracer incorporation was measured by beta counting.

2.4. Soluble factors: gene expression and protein detection

Cultured PBMC were harvested at different times and total cellular RNA was extracted, reverse transcribed and used in 30 cycles RT-PCR assays in the presence of primers specific for cytokine genes as previously described [14]. Secretion of soluble factors by cultured PBMC was assayed on supernatants harvested at different times by ELISA (Pharmingen, San Diego, CA).

2.5. Phenotyping of cells

Cells were phenotyped by staining with FITC or PE-conjugated mouse monoclonal antibodies (mAb) to human HLA-ABC, CD86, CD83, CD8, CD4 and CXCR3 (Becton Dickinson, San Diego, CA). T cells bearing receptors specific for $IM_{58–66}$ or Melan-A/MART-$1_{27–35}$ were detected by staining with HLA-A0201 tetramers containing the indicated peptides (Proimmune, Oxford, UK) [15]. Data are reported as percentages of cultured cells. Propidium iodide (final concentration: 160 ng/ml) was added to each sample to stain dead cells before acquisition by a flow-cytometer (FACScalibur) equipped with Cell Quest software (Becton Dickinson, San Diego, CA).

2.6. CTL induction experiments

Induction of HLA class I restricted CTL specific for $IM_{58–66}$ or Melan-A/MART-$1_{27–35}$ was addressed by tetramer staining and limiting dilution analysis (LDA) of CTL precursors (CTLp) frequency [15]. Briefly, PBMC or their indicated subsets were cultured in bulk cultures for tetramer staining or in multiple wells ($n = 84$) at three different responder concentrations, for LDA, in the presence of HLA class I restricted peptides (Neosystem, Strasbourg, France) (1 μg/ml for $IM_{58–66}$ and 10 μg/ml for Melan-A/MART-$1_{27–35}$) alone, or supplemented with either IRIV or L. Phenotyping and cytotoxicity assays were performed either on day seven of culture or on day 14 after one restimulation with autologous CD14+ cells preincubated for 2–3 h in the presence of the corresponding peptide. These peptide pulsed APCs were irradiated, and added to the cultures in the presence of IL-2 (20 units/ml final concentration). On day 10, cell cultures were supplemented with IL-2 for a second time at a final concentration of 100 units/ml. Cytotoxicity was assessed by ^{51}Cr release according to standard procedures. LDA wells were considered positive if specific lysis exceeded killing of targets pulsed with control peptides by at least 12% and was at least three standard deviations above spontaneous release values [15,16]. Specific CTLp frequency was evaluated by the Poisson formula [17].

3. Results

3.1. Adjuvant effects of IRIV on CD8+ CTL induction "in vitro"

PBMC from HLA-A0201+ healthy donors were cultured in the presence of IRIV and influenza matrix $IM_{58–66}$ peptide. After a one week incubation in the absence of exogenous IL-2, cells were collected and double stained with HLA-A0201/$IM_{58–66}$ tetramers and anti CD8 mAbs. Data from one representative experiment out of three are shown in Fig. 1. Barely detectable percentages of HLA-A0201/break $IM_{58–66}$ tetramer specific positive CD8+ cells could be observed in cultures stimulated with $IM_{58–66}$ alone (0.1%) or with $IM_{58–66}$ and liposomes (L: 0.2%). Instead, PBMC culture in the presence of soluble $IM_{58–66}$ and IRIV, resulted in powerful expansion of HLA-A0201/$IM_{58–66}$ tetramer positive cells within CD8+ population. Over 7% of all CD8+ cells showed evidence of specific tetramer binding.

Limiting dilution analysis of CTL precursor (CTLp) frequency demonstrated that upon one week culture, in cultures stimulated with $IM_{58–66}$ peptide in the presence of IRIV, 1/22.000 CD8+ T cells specifically recognized the target peptide. In contrast, no cytotoxicity was detectable in these short term cultures stimulated in the presence of $IM_{58–66}$ alone. When PBMC were stimulated with $IM_{58–66}$ and

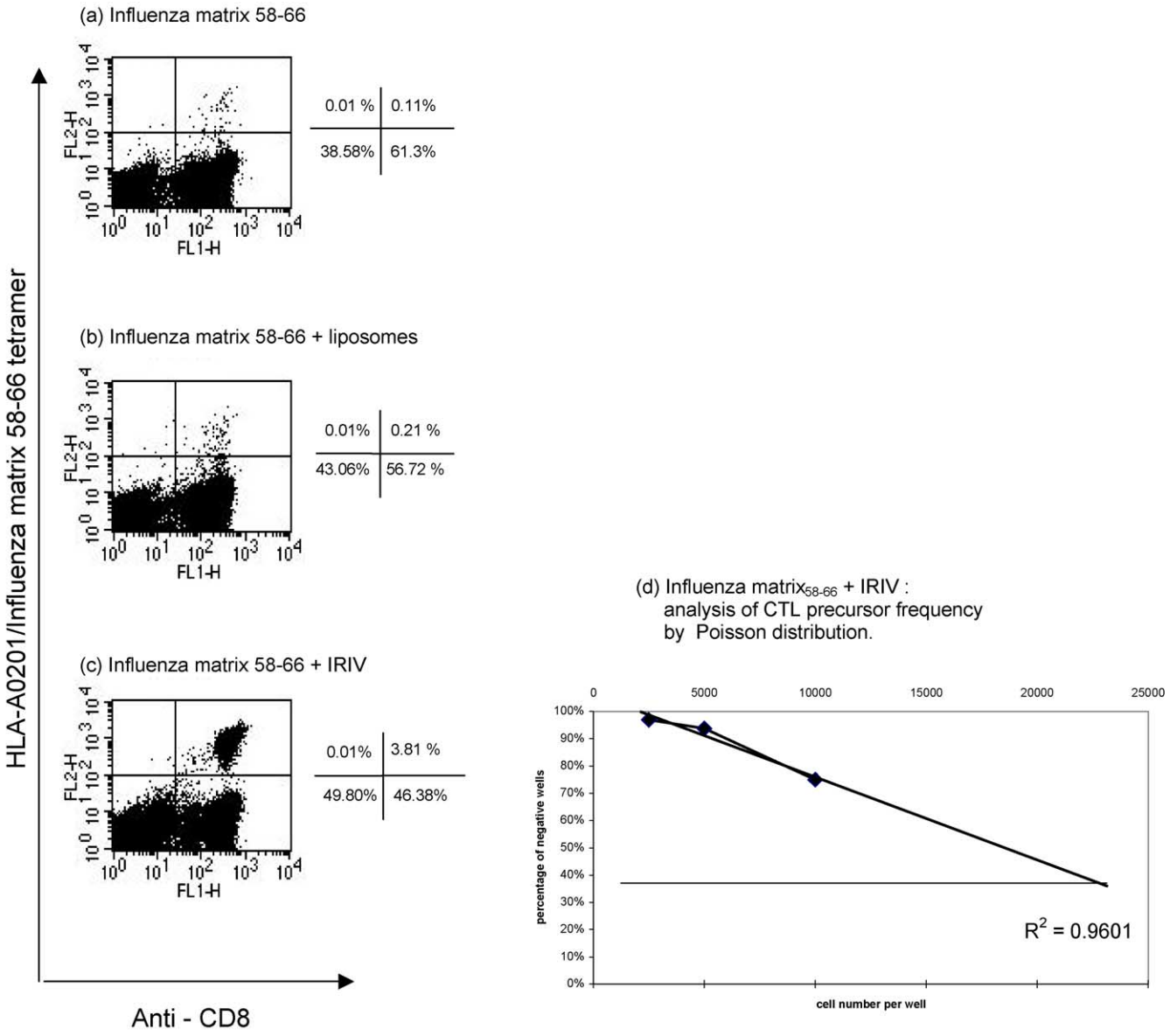

Fig. 1. IRIV adjuvance on CTL induction. PBMC from a healthy donor were cultured in the presence of $IM_{58–66}$ (a), $IM_{58–66}$ and control liposomes (b) or $IM_{58–66}$ and IRIV (c). After a 7 days culture, percentages of $IM_{58–66}$ specific CTL within cultured cells were quantified by HLA-A0201/$IM_{58–66}$ PE tetramer staining (fluorescence 2) and anti CD8 FITC staining (fluorescence 1). CTL precursor frequencies detected in $IM_{58–66}$ and IRIV stimulated cultures within the same experiment are shown in panel d.

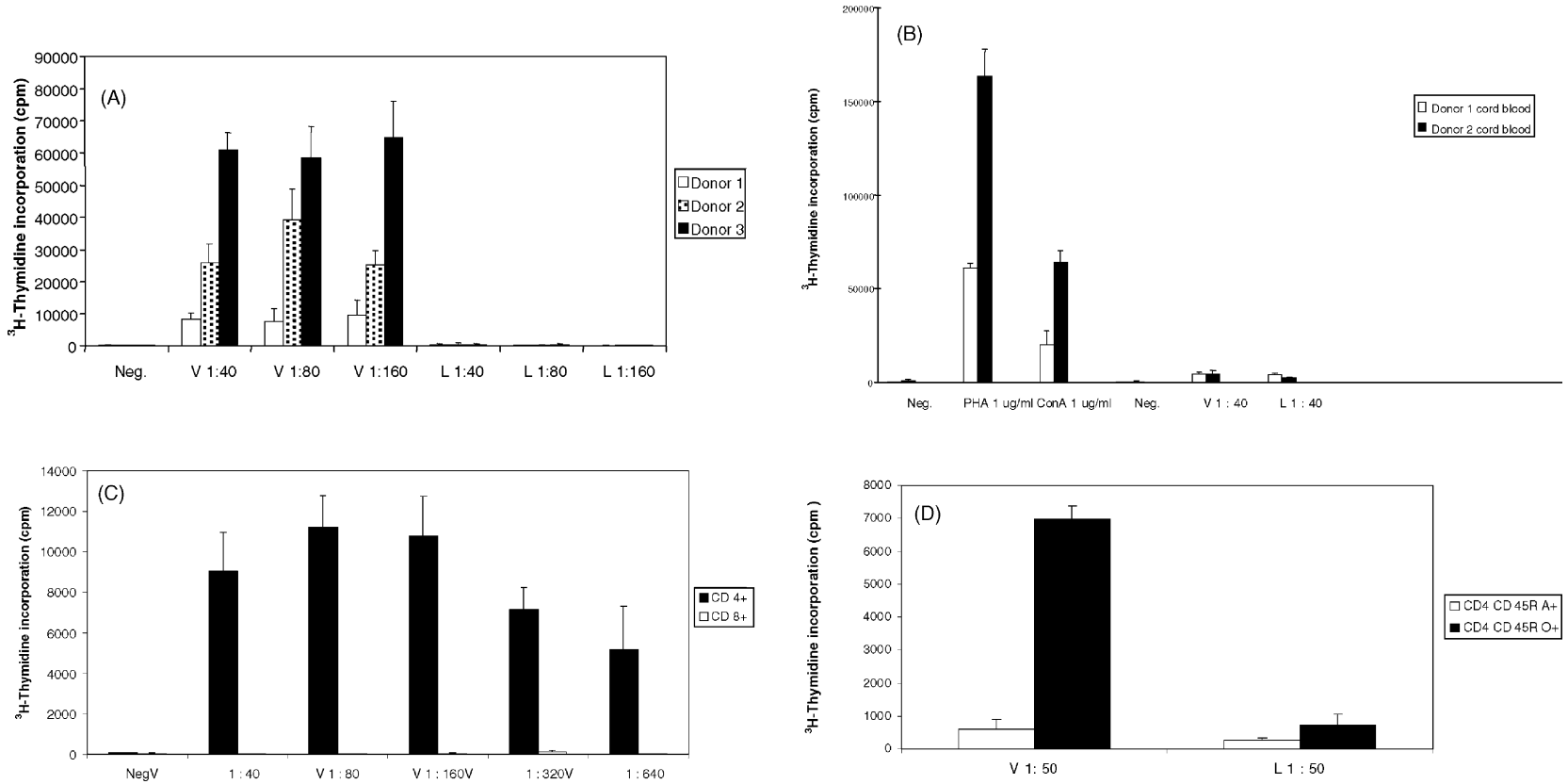

Fig. 2. IRIV induced antigen specific proliferation of CD4+ CD45RO+ cells. Panel A: PBMC from healthy donors ($n = 3$) were cultured in the absence of stimuli (Neg), in the presence of IRIV (V) and in the presence of control liposomes (L) at the indicated dilutions. Proliferation was measured on day six of culture by ^{3}H-thymidine incorporation. Panel B: cord blood mononuclear cells from two donors were cultured in the absence of stimuli (Neg) or in the presence of PHA, ConA, IRIV (V) or liposomes (L) at the indicated concentrations. Proliferation was measured on day three of culture for PHA and ConA cultures and on day six for IRIV and L stimulated cultures. Panel C: Purified CD4+ or CD8+ cells were cocultured with autologous irradiated PBMC in the absence of stimuli (Neg) and in the presence of IRIV (V) at the indicated concentrations. Proliferation was measured on day six of culture by ^{3}H-thymidine incorporation. Panel D: Purified CD4/CD45RA+ cells and CD4/CD45RO+ cells were isolated from PBMC of one healthy donor and cocultured with autologous irradiated PBMC in the presence of IRIV (V) or liposomes (L) at the indicated concentration. Proliferation was measured on day six of culture by ^{3}H-thymidine incorporation.

control L, 1/84 wells showed evidence of specific cytotoxic activity, a frequency below the treshold evaluable by Poisson distribution.

3.2. IRIV induce antigen specific proliferation of CD4+CD45RO+ cells

Since these data suggested a marked adjuvant effect of IRIV on CTL generation, we sought to clarify the underlying cellular mechanisms and we addressed the intrinsic immunostimulating capacities of IRIV, as suggested by a previous report [18]. PBMC from healthy donors were cultured in the presence of IRIV at different concentrations and proliferation was measured as ^{3}H-thymidine incorporation after 6 days incubation.

Cell proliferation could indeed be observed in PBMC cultures from all ($n = 10$) donors tested upon IRIV stimulation. One representative experiment is presented in Fig. 2, panel A. The extent of ^{3}H-thymidine incorporation was variable in cultures from different donors but no PBMC proliferation was detectable in cultures performed in the presence of the control L devoid of viral proteins.

To investigate the characteristics of the proliferation observed, we performed cord blood mononuclear cell cultures in the presence of IRIV or conventional mitogens. Both PHA and Con A induced marked cell proliferation. In contrast, IRIV only induced a marginal ^{3}H-thymidine incorporation in antigen naive cells, similar to that detectable in cultures performed in the presence of control L (Fig. 2, panel B). These data, from two different cord blood samples, supported the antigen specific nature of IRIV induced above described PBMC proliferation.

Experiments with purified responder cells from healthy donors' PBMC demonstrated that only CD4+ but not CD8+ T cells proliferated in the presence of IRIV (Fig. 2, panel C). Further dissection of CD4+ cells into CD45RA+ and CD45RO+ cells indicated that CD4/CD45RO+ T lymphocytes represented the main cell population responding to IRIV stimulation (Fig. 2, panel D). In keeping with data from cord blood experiments, this observation suggested that indeed antigen experienced cells proliferate upon IRIV stimulation.

3.3. IRIV stimulate a predominantly T helper 1 response

To further elucidate the characteristics of IRIV stimulated immune response we addressed cytokine gene expression and secretion profiles in cells and culture supernatants.

RT-PCR demonstrated the expression of IFN-γ, GM-CSF, TNF-α and IL-2 genes in PBMC upon IRIV stimulation (Fig. 3) whereas no expression of IL-4, IL-5 and IL-10 genes could be observed. Accordingly, ELISA assays demonstrated that IRIV stimulation induced the secretion of IFN-γ, GM-CSF and TNF-α with different kinetics. In contrast, as expected, IRIV did not promote IL-4 secretion (Fig. 4, panels A–D).

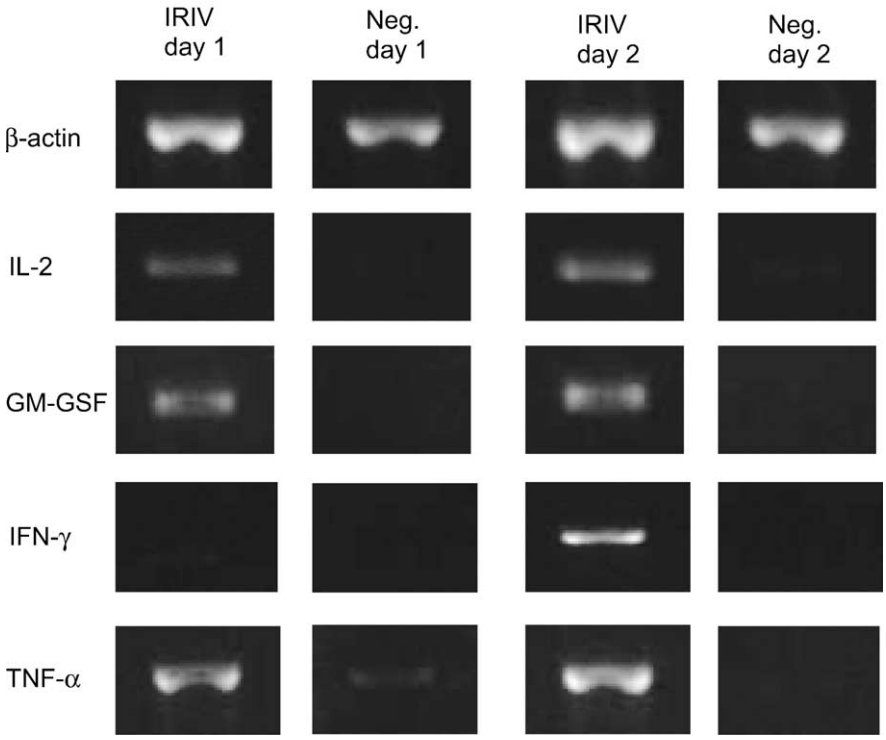

Fig. 3. Cytokine gene expression in IRIV stimulated PBMC. PBMC were cultured in the presence or absence of IRIV. On days one and two of culture, cells were harvested and total cellular RNA was extracted and reverse transcribed. The cDNAs thus obtained were tested in RT-PCR assays in the presence of primers specific for the indicated cytokine genes [14].

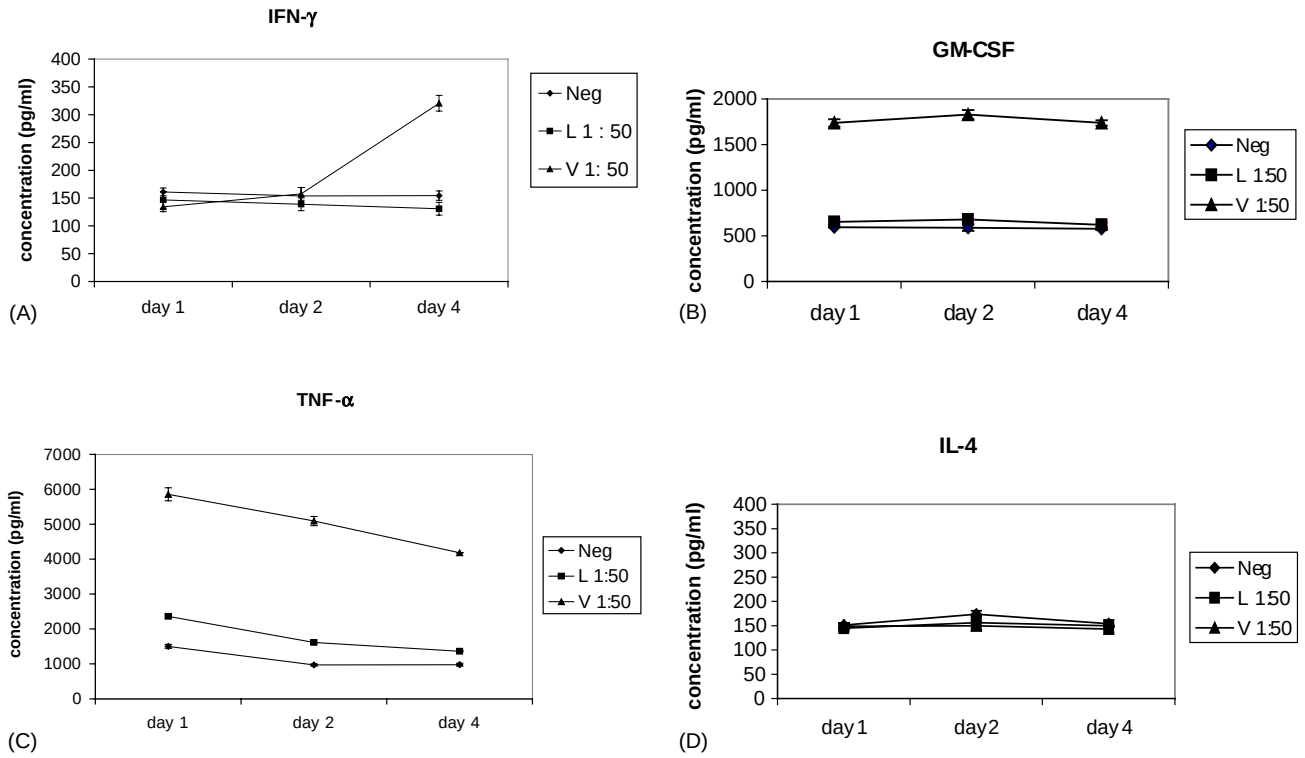

Fig. 4. Cytokine secretion in IRIV stimulated PBMC. PBMC from a healthy donor were cultured in the absence of stimuli (Neg) or in the presence of IRIV (V, 1:50 diluted) or control liposomes (L, 1:50 diluted). On day one, two and four, supernatants were harvested and the concentrations of IFN-γ (Panel A), GM-CSF (Panel B), TNF-α (Panel C) and IL-4 (Panel D) were determined by ELISA.

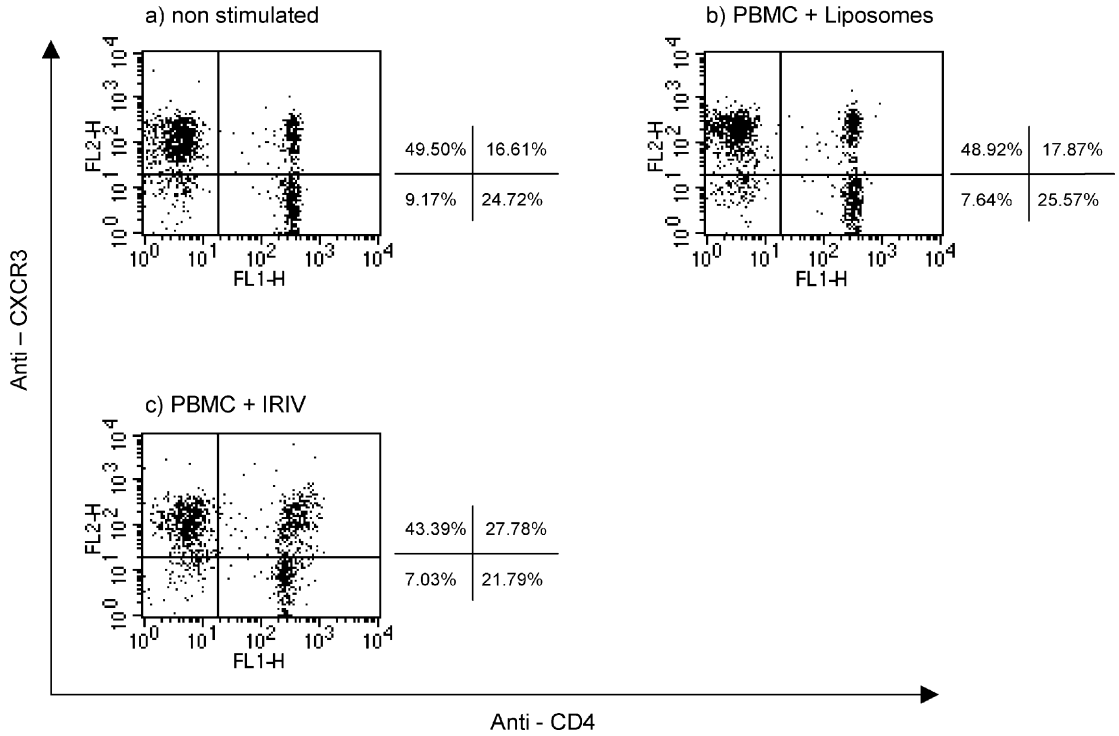

Fig. 5. Increased percentages of CXCR3 + CD4+ T cells in IRIV stimulated PBMC. Healthy donor's PBMC were cultured in the absence of stimuli (panel a), in the presence of liposomes (1:50 final dilution, panel b) or IRIV (1:50 final dilution, panel c). After 6 days of culture, cells were phenotyped for the expression of CXCR3 and CD4 by PE and FITC labelled mAbs, respectively.

Remarkably, discrete expression of defined chemokine receptors appears to represent a peculiar feature of specific CD4+ T cell subsets [19]. In particular, CXCR3 is a marker for Th1 immune responses and phenotyping of IRIV stimulated PBMC for this chemokine receptor may provide further indications on the nature of IRIV elicited immune responses. IRIV stimulated PBMC were double stained after 6 days of culture with anti CD4 and anti CXCR3 mAbs (Fig. 5). An increase in the percentage of CXCR3+ cells, as compared to unstimulated or L stimulated cultures was indeed observed within the CD4+ cell population.

3.4. IRIV adjuvant effects on the generation of IM 58-66 specific CD8+ CTL require live CD4+ T cells

The above data indicated that IRIV are powerful antigenic stimulators of CD4+ T helper 1 responses and suggested that these effects could play a major role in IRIV mediated adjuvance on CTL induction. To formally prove this hypothesis, we performed experiments where CTL induction was attempted in the presence of untreated or irradiated autologous CD4+ cells. In these experiments, a restimulation on day seven of culture with autologous CD14+, $IM_{58–66}$ pulsed, irradiated cells in the presence of rIL-2, as detailed in Section 2, was necessary for CTL induction.

Stimulation with soluble peptide only induced a barely detectable expansion of HLA-A0201/$IM_{58–66}$ tetramer positive CD8+ T cells, irrespective of the treatment of CD4+ T cells (Fig. 6 panel A). Similar results were obtained when liposomes devoid of viral proteins were added (data not shown).

In sharp contrast, $IM_{58–66}$ stimulation in the presence of IRIV (Fig. 6 panel B) resulted in the expansion of HLA-A0201/$IM_{58–66}$ tetramer positive CD8+ T cells when untreated CD4+ T cells were present in the cultures. In these conditions, over 14% of CD8+ cells showed evidence of $IM_{58–66}$ specificity. Pre-irradiation (30 Gy) of CD4+ cells added to the cultures, however, fully prevented IRIV mediated adjuvant effects on CTL induction.

3.5. Indirect effect on DC differentiation: induction of partial maturation

Prompted by the data on adjuvance in $IM_{58–66}$ CTL induction, we addressed these effects in a tumor specific setting, focusing on the HLA-A0201 restricted Melan-A/MART-

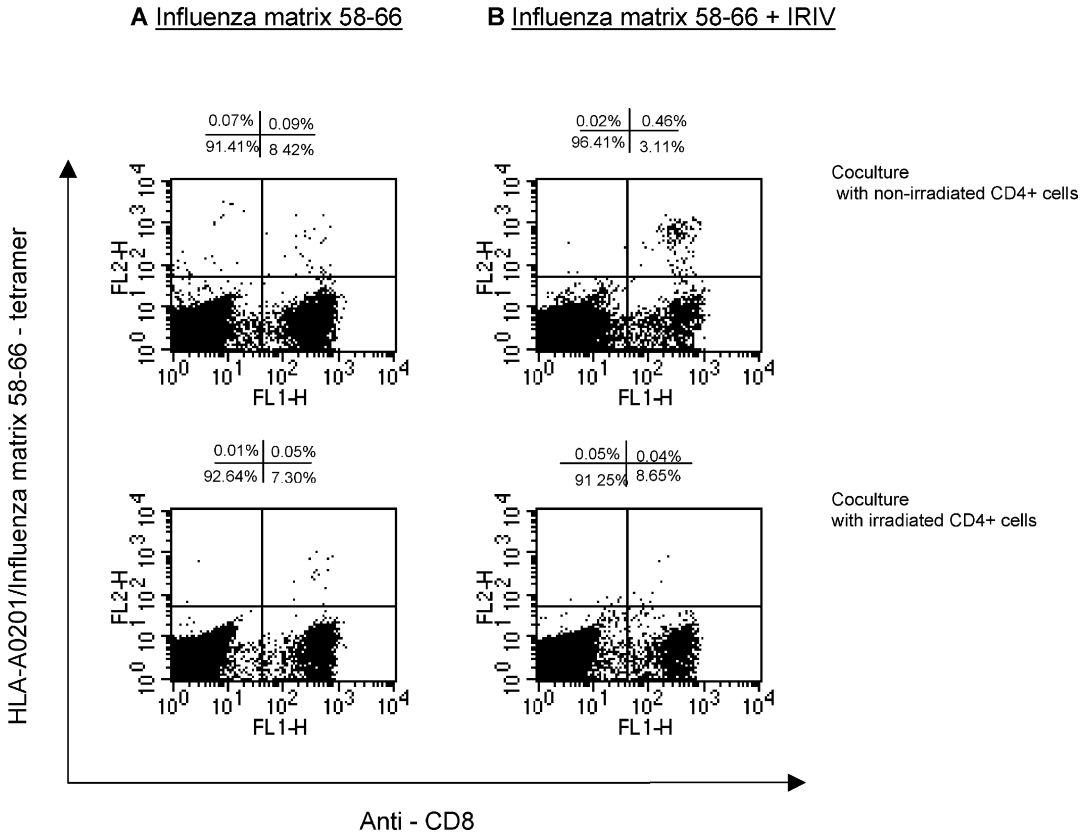

Fig. 6. IRIV mediated adjuvance in CTL induction requires live CD4+ T cells. CD8+ and CD14+ cells were cultured in the presence of autologous intact or irradiated CD4+ cells. These cultures were stimulated with influenza $matrix_{58–66}$ (1 μg/ml) alone (A) or supplemented with IRIV (1:50) (B). After 7 days of incubation, both cocultures were restimulated with irradiated influenza $matrix_{58–66}$ pulsed CD14+ cells and cultured for six further days in the presence of IL-2 (see materials and methods). Six days after restimulation, cultures were stained with HLA-A0201/Influenza $matrix_{58–66}$ PE specific tetramers and anti CD8 FITC mAbs.

$1_{27–35}$ melanoma associated epitope. In this case optimal CTL induction has been shown to rely on the use of adequate antigen presenting cells (APC), particularly mature dendritic cells (mDC) [20].

First, we tested whether IRIV could induce DC maturation in vitro. In our hands direct addition of IRIV or L to iDC did not result in upregulated expression of maturation markers (data not shown).

On the other hand, consistent with the presence of discrete cytokines (see above), incubation of iDC with supernatants from IRIV stimulated PBMC cultures resulted in an increased expression of CD83, CD86 and HLA class I molecules. In a typical experiment, mean fluorescence intensities were upregulated from 9, 49 and 407 to 33, 242 and 634, respectively, as compared to incubation in the presence of supernatants from L stimulated cultures. However, no expression of CCR7 could be induced (data not shown). Taken together these data suggest that partial maturation of iDC can be induced by culture supernatants of IRIV stimulated PBMC.

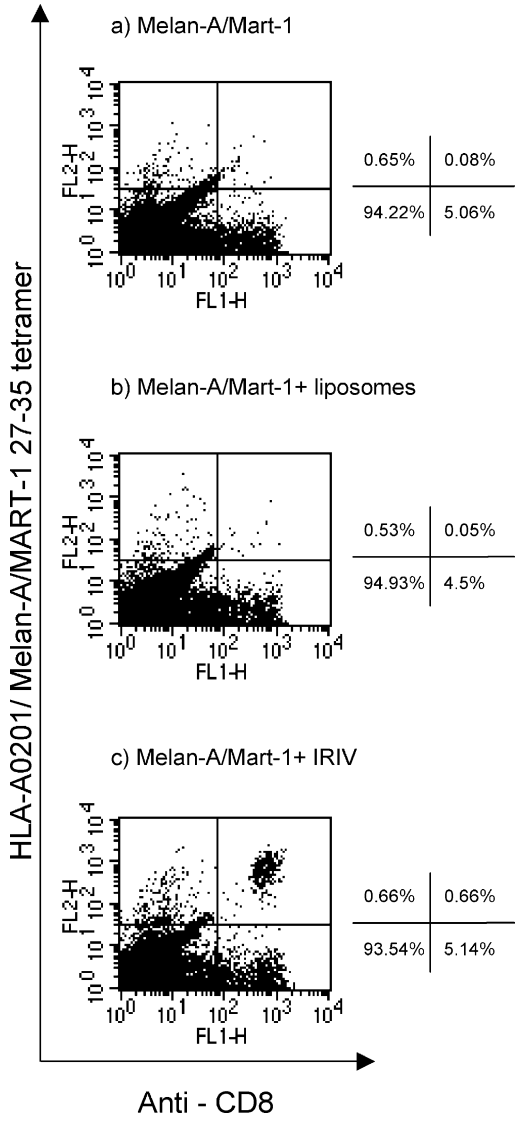

Fig. 7. Adjuvant effects of IRIV in the induction of tumour associated antigen specific CTL. CD14 negative cells from a healthy donor PBMC were cocultured with autologous iDC in the presence of Melan-A/Mart-$1_{27–35}$, alone (a) or supplemented with either control liposomes (b) or IRIV (1:50, c). On day seven of culture, cells were restimulated with Melan-A/MART-$1_{27–35}$ pulsed iDC and cultured for six further days (see material and methods). On day seven after restimulation, cells were stained with FITC conjugated anti-CD8 and PE conjugated HLA-A0201/Melan-A/MART-$1_{27–35}$ tetramers.

3.6. IRIV effects on the induction of Melan-A/Mart-$1_{27–35}$ specific CTL

In order to address IRIV effects on the induction of Melan-A/MART-$1_{27–35}$ specific CTL we cultured CD14- cells together with iDC in the presence of peptide and IRIV, peptide and L and in the presence of peptide alone. After one restimulation with Melan-A/MART-$1_{27–35}$, pulsed and irradiated autologous iDC in the presence of rIL-2 (see Section 2) cells were collected and double stained with HLA-A0201/Melan-A/MART-$1_{27–35}$ tetramers and anti CD8 mAbs. Data from one representative experiment, shown in Fig. 7 indicate that hardly any expansion of HLA-A0201/Melan-A/MART-$1_{27–35}$ tetramer positive CD8+ cells was detectable in cultures performed in the presence of peptide alone or supplemented with L. On the other hand, cell culture in the presence of Melan-A/MART-$1_{27–35}$ and IRIV resulted in marked expansion of HLA-A0201/Melan-A/MART-$1_{27–35}$ tetramer positive cells within the CD8+ population. Over 12% of all CD8+ cells showed evidence of specific tetramer binding.

4. Discussion

Immunopotentiating reconstituted influenza virosomes (IRIV) are liposomal formulations used as adjuvants in vaccine preparations. IRIV adjuvance has been demonstrated to enhance humoral immune responses [21]. However, little is known regarding the action of IRIV on cell mediated immune responses. Here we addressed the effects of IRIV on the induction of antigen specific cytotoxic T lymphocytes (CTL) in vitro by using as model antigens the HLA-class I restricted peptides $IM_{58–66}$ and the tumor associated differentiation antigen Melan-A/MART-$1_{27–35}$.

PBMC culture in the presence of $IM_{58–66}$ and IRIV resulted in marked expansion of peptide specific CTL within the CD8 T cell population as compared to cultures performed in the presence of peptide alone or supplemented with control liposomes. Remarkably these effects were observed in the absence of exogenous IL-2 supplementation.

To obtain an insight into the mechanisms underlying IRIV elicited adjuvance on CTL induction we addressed their stimulatory effects on PBMC. Indeed, IRIV have been shown to induce proliferation in healthy donors' PBMC [18]. However, the nature of this blastogenic response,

whether antigenic or mitogenic, has not been characterized, nor was the identity of stimulated cells addressed. Our assays demonstrate that IRIV induce proliferation of antigen experienced CD4 T cells (CD4 + CD45RO+). The antigenic nature of this stimulation is further supported by the absence of significant proliferation in cord blood mononuclear cells cultured in the presence of IRIV as compared to those cultured in the presence of liposomes devoid of viral proteins.

We then investigated the cytokine profile induced by IRIV stimulation of PBMC. Both RT-PCR and ELISA results demonstrated that IRIV induced increased expression of T helper 1 cytokines such as IFN-γ, GM-CSF and TNF-α in PBMC. In addition, IRIV resulted in increased percentages of CD4+ T cells expressing CXCR3, a chemokine receptor associated with discrete inflammatory Th-1 type reactions [19]. Thus, both, cytokine gene expression and secretion data and CXCR3 expression concur in supporting the notion of a prevailing T helper 1 stimulation by IRIV. Remarkably, consistent with our data, peripheral blood T cells expressing CXCR3 have been shown to be mostly CD45RO+ [19].

To clarify the role of CD4 T cell proliferation in IRIV mediated CTL adjuvance, we cocultured CD8+ T cells with CD14+ cells and with either untreated or irradiated CD4+ T cells in the presence of IRIV and $IM_{58–66}$. Increased percentages of specific CTL were detectable in the presence of untreated but not of irradiated CD4+ T cells. These findings indicate that IRIV CTL adjuvance requires intact CD4+ T cells.

CD4+ T cell mediated helper effects on CTL induction have been suggested to rely on the production of cytokines enhancing activated CTL proliferation [22]. Clearly this mechanism could play a role in IRIV adjuvance on CTL generation. Alternatively, CTL “help” related to promotion of APC activation, possibly through CD40 triggering, has also been proposed [22–24]. Indeed, although limited expression of CD40L was detectable in IRIV stimulated cells (data not shown), culture supernatants were able to induce at least partial maturation of iDC, as related to upregulation of HLA, costimulatory molecules and CD83 expression. IRIV induced IFN-γ and TNF-α secretion can reasonably be held responsible for these effects.

CTL induction is likely to play a critical role in immunity against defined infectious agents. Moreover, it represents the main objective of active specific cancer immunotherapy. Thus, we attempted to extend the validity of our findings on IRIV induced CTL adjuvance to this area.

Our results demonstrate IRIV adjuvance in the induction of CTL specific for Mart-1/Melan-$A_{27–35}$ tumor associated differentiation antigen. Further investigation related to additional tumor associated antigens is warranted to support the use of IRIV in tumor immunotherapy protocols.

Previous research suggested that the role of IRIV in CTL induction might mostly reside in their capacity to protect encapsulated antigens from peptidases/proteases present in the tissue microenvironment or to deliver antigens intracellularly according to different modalities [7–11,25,26]. These properties are common to different liposomal preparations. Our results indicate that, regardless of these features, IRIV “per se” are endowed with a high adjuvant capacity for CTL induction. Most importantly our data suggest that this capacity is largely attributable to their ability to antigenically stimulate CD4+ T cells. Thus, a “secondary” CD4+ T cell response is efficiently providing “help” for the induction of class I restricted CD8+ effector T cells.

Acknowledgements

This work was partially supported by a grant from the Kommission für Technologie und Innovation (Bern, Switzerland) to MH. Critical reading of the manuscript by Anca Reschner and Chantal Feder is gratefully acknowledged.

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