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ICAM-1 overexpression counteracts immune-suppression by tumour cell-derived PGE_2 to restore CTL function

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ABSTRACT

Tumour-infiltrating cytotoxic T lymphocytes (CTLs) play a key role in tumour killing. However, many cancers adopt various strategies to induce immunosuppression. Priming of naïve $CD8^+$ T cells to become CTLs occurs via cognate interactions of the T cell receptor (TcR) and CD28 with tumour-derived peptide epitopes expressed on major histocompatibility complex (MHC) class I molecules and CD80/CD86 on T cells and antigen-presenting cells (APCs) respectively. Here we report that, in the absence of CD80/CD86 expression by renal carcinoma (Renca) cells, expression of intercellular adhesion molecule-1 (ICAM-1) by Renca cells provides a potent alternative co-stimulation to a tumour-specific $CD8^+$ T cells causing them to produce interferon gamma (IFN-γ) which is crucial for the further up-regulation of ICAM-1 on tumour cells. We have shown that overexpression of cyclooxygenase-2 (COX-2), by Renca cells (Renca-T3), results in increased levels of prostaglandin (PG) E_2 production, which can directly suppress anti-tumour $CD8^+$ T cells resulting in loss of CTL function *in vivo* and cause metastases to the tumor-draining lymph nodes (TDLNs). Significantly, our data also show that overexpression of ICAM-1 on Renca-T3 cells can counteract the immune-suppressive effect of PGE_2 and restore CTL responses.

Introduction

Anti-tumour $CD8^+$ cytotoxic T lymphocytes (CTLs) are capable of producing interferon gamma (IFN-γ) and cytolytic enzymes and are crucial for cancer regression[1]. Priming naïve $CD8^+$ T cells to become CTLs requires two signals; the first comes from T cell receptor (TcR) interaction with peptide epitope presented by major histocompatibility complex (MHC-I) molecules on antigen-presenting cells (APCs). The second signal was long thought to be solely provided by the interaction of T cell-expressed CD28 with the classical co-stimulatory CD80/CD86 molecules on APCs. However, CD80- and CD86-mediated co-stimulation is not provided by most tumour cells. Thus, the direct interaction between naïve $CD8^+$ T cells and tumour cells often leads to tolerance induction. In the absence of CD80/CD86[2], intercellular adhesion molecule-1 (ICAM-1) interaction with lymphocyte function associated antigen-1 (LFA-1), expressed by T cells, can provide a co-stimulatory signal resulting in the formation of CTLs[3,4].

ICAM-1 is a member of immunoglobulin superfamily expressed by many cells including various tumour cells[5]. The main role of ICAM-1 is to firmly arrest leukocytes and facilitate extravasation through

the blood vessels to sites of inflammation. However, ICAM-1 also enables T cells to adhere to other cells including APC, endothelial cells, and to normal and tumour cells[6]. In some cancer patients elevated levels of soluble (s) ICAM-1 of around 3 to 5-fold are often found. However, Circulating sICAM-1 is shown to block LFA-1; thus promoting tumour growth and angiogenesis[7]. In contrast, ICAM-1 and/or ICAM-2 negative pancreatic cancer cells are resistant to killing by γδ T cells. However, such resistance to killing can be reversed by re-expressing ICAM-1 or ICAM-2[8].

Despite the induction of tumour-specific CTL responses by alternative co-stimulation pathways, tumours still develop various strategies to escape anti-tumour immune responses, such as the over-production of prostaglandin (PG) E_2 as a result of the up-regulation of the cyclooxygenase-2 (COX-2) gene[9,10]. PGE_2 is a known immune modulator which is able to maintain dendritic cells (DC) in an immature state by: increasing the levels of IL-10 within the tumour microenvironment; enhancing Th17 T cell responses, and recruiting myeloid-derived suppressor cells (MDSC)[11]. In addition, coupling PGE_2 with cognate receptors on T cells can increase cyclic adenosine-monophosphate (cAMP) within T cells which, consequently, reduces T cell proliferation and effector function[12]. Moreover, PGE_2 decreases IFN-γ production by T cells. IFN-γ is shown to be involved in enhancing tumour immunogenicity. PGE_2 can indirectly affect the expression of ICAM-1 by preventing IFN-γ mediated up-regulation of ICAM-1. Therefore, we hypothesized that over-expressing ICAM-1 on COX-2-overexpressing Renca-T3 cells would counteract the immunosuppressive effect of PGE_2 on tumour-specific $CD8^+$ T cells.

To test this hypothesis, and to explore the efficacy of ICAM-1 in providing co-stimulatory signals to tumour-specific $CD8^+$ T cells, our lab adapted a well-established murine renal carcinoma (Renca) model to generate cells that not only express the haemagglutinin (HA) protein from influenza virus A/PR/8/H1N1 (PR8) as a neo-tumour-specific antigen (Renca-HA;[3]), but which also over-express COX-2, resulting in the over-production of PGE_2 (Renca-T3;[12]).

Materials and methods

Mice

6 to 8 wk old $Thy1.1^{+/+}$ $CL4^{+/-}$ TcR transgenic BALB/c mice (CL4 mice;[13]) were bred and housed under specific pathogen-free conditions at the University of Bristol Animal Services Unit. All experimental procedures were conducted in accordance with U.K. Home Office guidelines.

Murine renal carcinoma (Renca) tumour cells

Renca-wild type (WT) were cultured in complete media consisting of RPMI (1640) (Sigma- Aldrich, Poole, UK) supplemented with 10 % (v/v) foetal calf serum (FCS), 2 mM L-glutamine, 50 U/ml penicillin/streptomycin (Life technologies, Paisley, UK), 5 $\times 10^{-5}$ M 2-mercapto-ethanol (Sigma-Aldrich, St. Louis, USA). The Renca-HA cells used throughout this study expressed low levels of HA, (Renca-HA^{lo}; as described in[3]), and were grown in complete medium supplemented with 100 μg/ml geneticin (G418; Sigma-Aldrich), and for Renca-T3 medium was additionally supplemented with puromycin (1 μg/mL; Biomatik). Renca-HA/ICAM-1 and Renca-T3/ICAM-1 cells were generated by transfecting tumour cells with 1.5μg of the ICAM-1-expressing vector: pIREShyg (a kind gift from Prof. Adrian Whitehouse at Leeds University) and ICAM-1 cDNA (Sino Biological Inc. Beijing, China) by lipofection. All Renca cells were then left to grow in complete media additionally supplemented with 250 μg/ml of hygromycin B. Resistant colonies were picked and examined for ICAM-1 expression by flowcytometry. Both HA and ICAM-1 expression were routinely checked during this study.

Enrichment and proliferation of CL4 $CD8^+$ T cells

Naïve CL4 $CD8^+$ T cells were purified from peripheral lymphoid tissues isolated from CL4 TcR transgenic mice by magnetic-activated cell sorting (MACS) according to the manufacturer's instructions (Miltenyi Biotec Ltd., Bisley, UK). In some instances, naïve CL4 T cells were labeled with 5 μM carboxyfluorescein succinimidyl ester (CFSE; BioLegend, San Diego, USA). MACS-purified CL4 T cell proliferation was detected either; by a ^{3}H-thymidine-based proliferation assay, whereby 1 μCi ^{3}H-thymidine was added in the last 8 hours and the proliferation was measured by thymidine incorporation (counts per minute; cmp) (Amershan Life Science, London, UK), or by a CFSE-based proliferation assay whereby CL4 proliferation was detected by the loss of CFSE expression. Naïve CL4 T cells were activated with; 10 μg/ml of anti-CD3 mAbs plus either; 5 μg/ml of anti-CD28 mAbs, or 3 μg/ml of recombinant (r) ICAM-1, or populations of γ-irradiated Renca cells. Renca cells were irradiated with 9600 RADs using a Cs^{137} source of γ-irradiation (RX30/55; Gravatom Projects, Grosport, UK).

Flow cytometry

Activated CL4 T cells were either left untreated, or stimulated with 1 μg/ml K^dHA peptide and 1 μg/ml GolgiPlug (BD Bioscience, San Diego, USA), before staining with live/dead aqua and fluorochrome-conjugated monoclonal antibodies (mAbs) for surface staining or they were first permeabilized and then stained intracellularly for IFN-γ.

Statistical analyses

P values were calculated by one-way ANOVA followed by either Dunnett's or Bonferroni's multiple comparison tests using the Prism 5.03 software (GraphPad Software, Inc.).

Results

Characterization of ICAM-1 and LFA-1 expression by naïve CL4 CD8$^+$ T cells

To investigate the role of CD8$^+$ T cells in anti-tumour immunity, our lab utilized CL4 TcR transgenic mice in which virtually all the CD8$^+$ T cells express the Vα10/Vβ8.2 TcR transgene[13]. These CL4 T cells are able to recognize the dominant K^d-restricted epitope of the Influenza virus A/PR/8 haemagglutinin (HA) protein (K^{d}HA$_{[IYSTVASSL]}$) expressed by HA-transfected Renca-HA cells[3]. Naïve CL4 CD8$^+$ T cells were purified from the total cells harvested from lymphoid tissues of CL4 mice using the MACS purification technique. This process routinely results in >98% pure CD8$^+$ T cells (Figure 1A). ICAM-1 has a central role in naïve CD8$^+$ T cell priming[3], and acts as a CD28-independent co-stimulator[2]. ICAM-1 interacts with LFA-1 expressed by T cells which is crucial for homotypic T cell aggregation and communication[14]. Therefore, MACS-purified naïve CL4 CD8$^+$ T cells were stained for ICAM-1 and LFA-1 expression. FACS plots confirm the expression of both ICAM-1 and LFA-1 by naïve CL4 T cells (Figure 1B & C).

Is ICAM-1 a potent alternative co-stimulatory molecule?

To evaluate the ability of Renca-HA cells, to prime naïve CL4 CD8$^+$ T cells, both the HA low-expressing Renca-HA cells[3], and control Renca-WT cells were used as APC to prime naïve CL4 T cells in a standard ^{3}H-thymidine incorporation T cell proliferation assay. Whilst naïve CL4 T cells did not proliferate in response to control irradiated Renca-WT APCs *in vitro*, significant levels of CL4 T cell proliferation was observed when cultured with Renca-HA cells as APC (Figure 2A). These data, therefore, suggest that Renca-HA cells are not only able to provide CL4 T cells with signal one but also co-stimulatoty signal two. However, we know that although Renca-HA cells lack CD80/CD86; they do express ICAM-1 (Figure 2B).

To determine if the co-stimulation in our system occurs solely via ICAM-1, anti-ICAM-1 blocking mAbs were added to the CL4 T cells/Renca-HA co-culture. In Figure 3, bar charts show that not only does blocking ICAM-1 significantly decrease CL4 T cell proliferation *in vitro* (A), IFN-γ production is also inhibited; as evidenced by the fact that CL4 T cells were unable to produce IFN-γ compared to the non-treated co-culture (Figure 3B: compare 2.7% with 59%). However, the data show that for both CL4 T cell proliferation in response to classical (anti-CD3 mAbs + anti-CD28 mAbs), and alternative (anti-CD3 mAbs + rICAM-1) priming, only the presence of plate-bound rICAM-1 and not soluble (s) rICAM-1 resulted in enhancement of CL4 T cell proliferation. In addition, treating naïve CL4 T cells with rICAM-1 in solution for an hour before the beginning of the culture is not sufficient to induce the proliferation to a level similar to that achieved using plate-bound rICAM-1 (Figure 3C). Furthermore, there is a positive correlation between the cell surface expression of ICAM-1 on Renca cells and CL4 T cell proliferation as shown in (Figure 3D & E); the more cell surface ICAM-1 that is expressed the more CL4 T cell proliferation is detected. Importantly, these differences in proliferation were not due and changes in the level of HA expression, as after overexpressing ICAM-1, HA expression remained the same for all clones; regardless of the levels of ICAM-1 (Figure 3E).

Can ICAM-1 counteract the inhibitory effect of PGE_2?

Although the basal level of ICAM-1 expression by Renca-HA cells is low, such low level expression is enough to provide sufficient alternative co-stimulation to prime naïve CL4 CD8$^+$ T cells *in vitro*. However, despite this, Renca-HA cells continue to grow *in vivo*. Overexpression of COX-2 by Renca-T3 cells resulted in elevated PGE_2

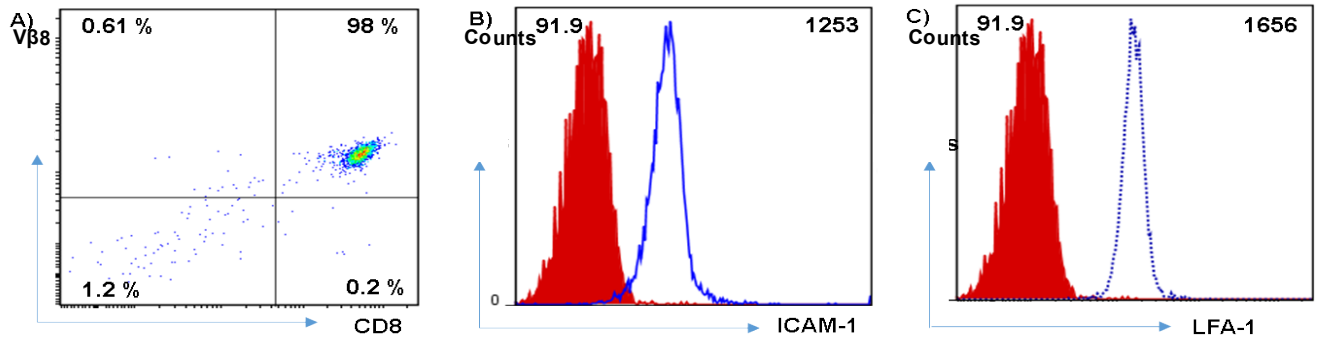

Figure 1: TcR, ICAM-1 and LFA-1 expression by naïve CL4 CD8$^+$ T cells.
A) Spleen and lymph nodes were harvested from CL4 TcR transgenic mice and single cell suspensions were generated. CL4 cells were enriched for CD8$^+$ T cells using anti-CD8 magnetic beads and were stained with anti-CD8α-APC and anti-Vβ8-FITC mAbs for flow cytometric analyses. The numbers in the top right corner of FACS plot (A) represent the percentage of Vβ8$^+$CD8$^+$ double positive T cells. MACS-purified CL4 CD8$^+$ T cells were stained with either (B) anti-CD54-FITC mAbs for ICAM-1, or with (C) anti-CD11a-Alex Fluor 488 mAbs for LFA-1. In both (B) and (C), the filled histograms show the isotype control, whereas the solid lines represent the stained cells. Numbers represent the mean of fluorescence intensity (MFI). Data are representative of more than 15 separate experiments.

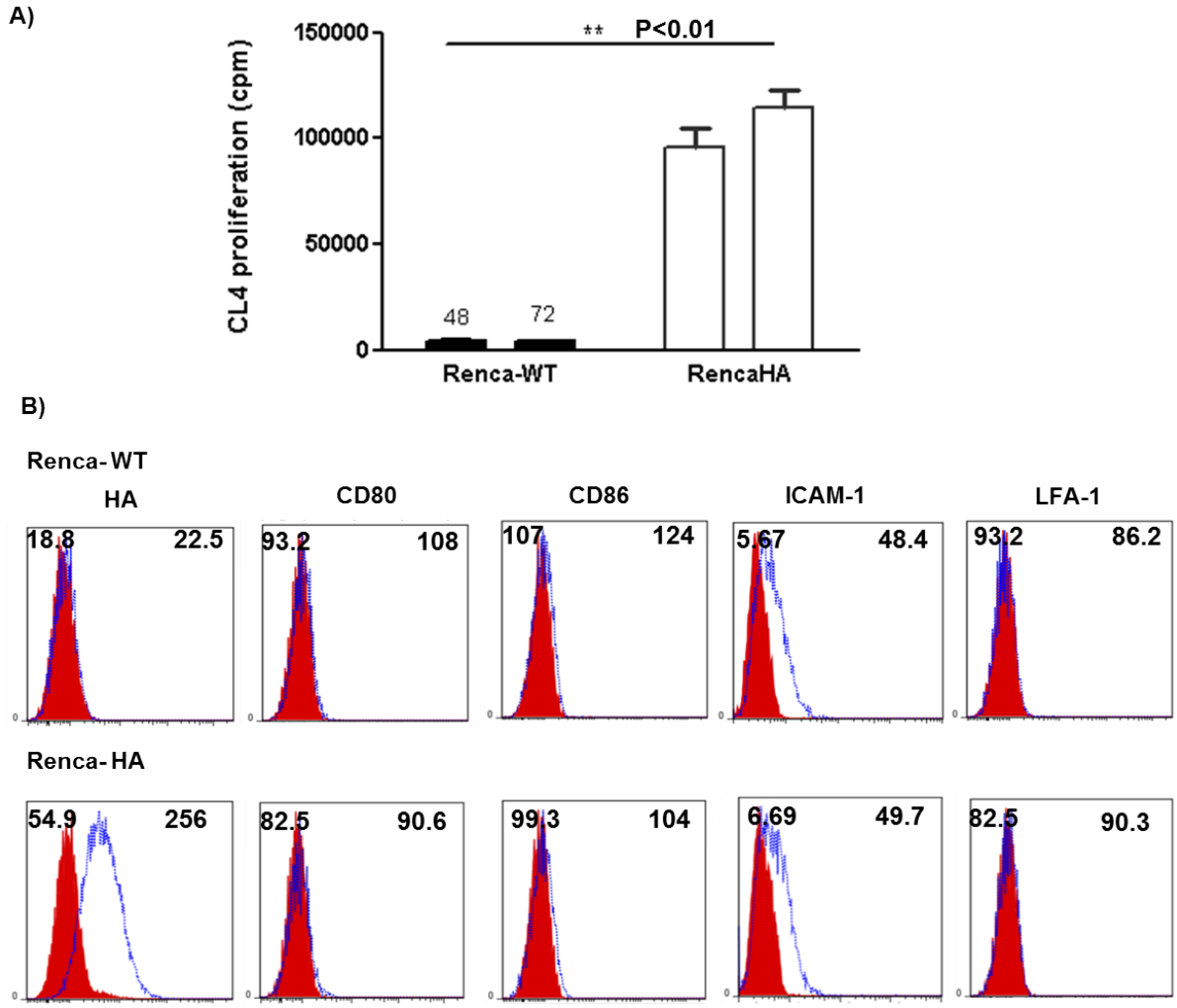

Figure 2: CL4 CD8⁺ T cell proliferation in response to Renca-WT or Renca-HA cells.

A) MACS-purified naïve CL4 CD8⁺ T cells were co-cultured with either irradiated Renca-WT or Renca-HA cells as APCs at a 1:1 effector:target ratio, for 48 and 72 hours. Plates were pulsed with 1 µCi of ^{3}H-thymidine in the last 8 hours. After which, CL4 T cells were harvested and the level of ^{3}H-thymidine incorporation was measured. Bar charts show counts per minute (cpm). Error bars represent mean ±SEM. Data are representative of 3 separate experiments. Statistical analyses were carried out using one-way ANOVA followed by Dunnett's multiple compression test. ** P<0.01

B) Renca-WT and Renca-HA cells were harvested and stained with live/dead aqua and fluorochrome-conjugated mAbs: anti-CD80-FITC, anti-CD86-Alex Fluor 647, anti-CD54-FITC and anti-CD11a -Alex Fluor 488. HA expression was examined using 2-step FACS staining in which Renca cells were incubated first with anti-HA-biotin (Bio), followed by Streptavidin (SA)-PE. Filled lines represent the isotype control, whereas the dotted lines show the indicated surface molecule. Numbers represent the mean of fluorescence intensity (MFI). Data are representative of 3 separate experiments.

production; which has been shown to inhibit anti-tumour responses *in vitro* and *in vivo*[12,15]. To compare the effect of PGE_2 on the proliferation and the production of IFN-γ by $CD8^+$ T cells under classical (anti-CD28), and alternative (rICAM-1), co-stimulation pathways, CFSE-labeled CL4 T cells were primed with either; anti-CD3mAbs + anti-CD28 mAbs, or with anti-CD3 mAbs + plate-bound rICAM-1, in the presence or absence of 1 µM PGE_2. In the absence of any PGE_2, the FACS plots shown in Figure 4A&B; top row, reveal that around 75% of anti-CD3 mAb-treated CL4 T cells receiving alternative co-stimulation through plate-bound rICAM-1 underwent two or three rounds of division; (each peak showing successive loss of CFSE refers to one round of division). This is compared with only 55% of anti-CD3mAbs-treated CL4 T cells receiving classical costimulatory signaling through anti-CD28 mAbs. This decrease in the proliferation is also associated with a reduction in the number of IFN-γ producing CL4 T cells (Figure 4A&B; bottom row). However, in the presence of PGE_2, there is a decrease in IFN-γ-producing CL4 T cells of nearly 4-fold amongst those cells receiving classical anti-CD28 costimulation; compared to a 2-fold decrease in IFN-γ production among CL4 T cells receiving alternative costimulation via rICAM-1 (Figure 4A&B; bottom row).

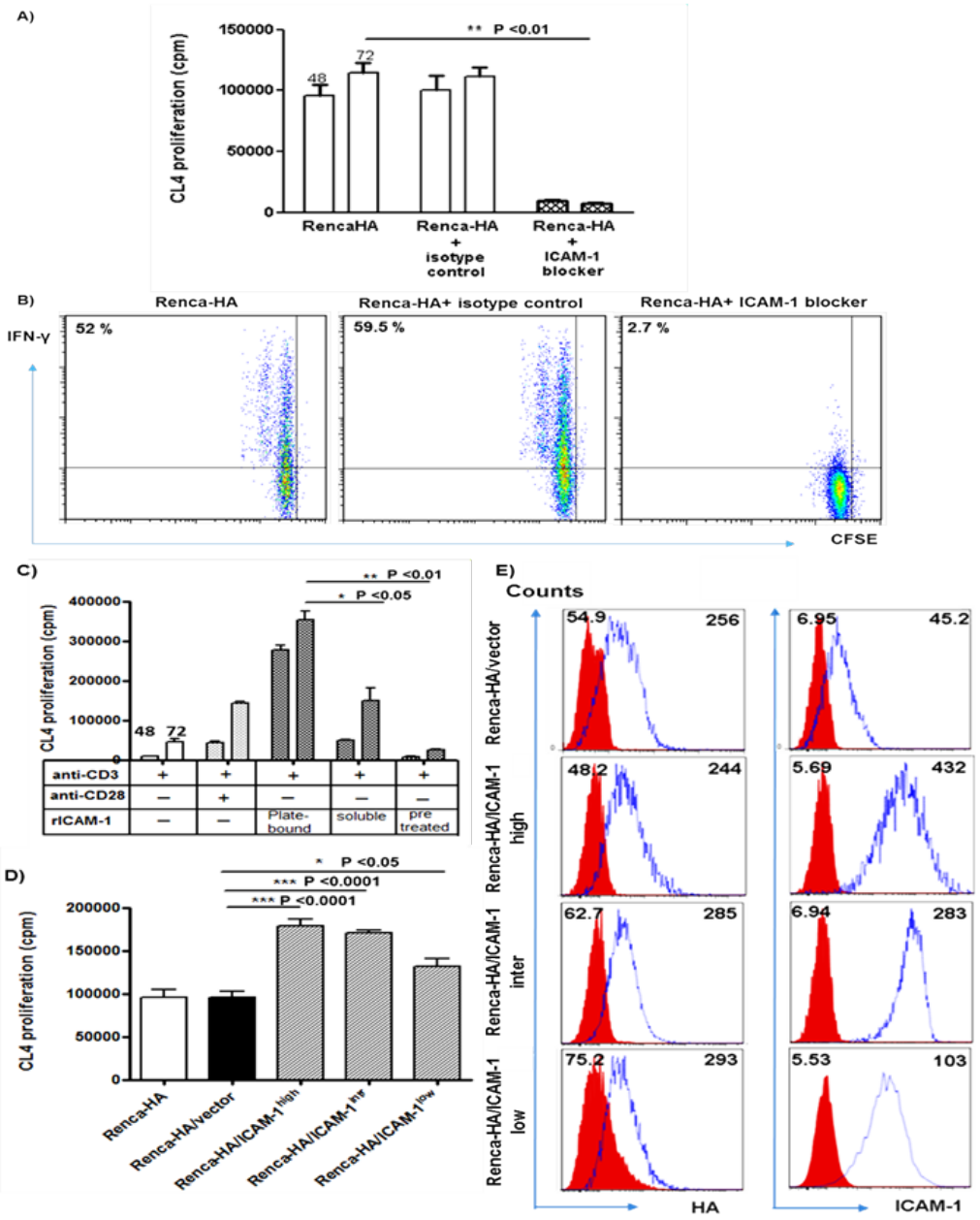

Figure 3: Proliferation and IFN-γ production by CL4 CD8⁺ T cells in the presence or absence of anti-ICAM-1 blocking mAbs.

(A) MACS-purified naïve CL4 CD8⁺ T cells were co-cultured for with irradiated Renca-HA at a 1:1 effector: target ratio in the absence or presence of 10 µg/ml goat anti-mouse ICAM-1/CD54 antibody, or with goat IgG isotype control. During the final 8 hours, cultures were pulsed with 1 µCi ^{3}H-thymidine. CL4 cells were harvested and analysed for ^{3}H-thymidine incorporation. Bar charts show counts per minute (cmp). Error bars are mean ±SEM. Data are representative of 3 separate experiments. Statistical analyses were carried out using one way ANOVA followed by Dunnett's multiple comparison test. ** P<0.01.

(B) 1x10^{6} MACS-purified naïve CL4 CD8⁺ T cells were co-labelled with CFSE and cultured with 1x10^{5} irradiated Renca-HA cells in the absence or presence of 10 µg/ml goat anti-mouse ICAM-1 blocking mAbs, or with goat IgG isotype control mABs, for 72 hours. CL4 T cells were then collected re-stimulated with 1 µg/ml of K^{d}HA and Golgi plug for four hours, and then stained with live/dead aqua before being permeabilized and stained intracellularly with anti-IFN-γ-APC mAbs. FACS plots show the expression of CFSE *versus* the production of IFN-γ by CL4 T cells. The numbers in each FACS plot show the percentage of proliferated CL4 T cells that are also IFN-γ-positive (top left corner). Data are representative of 3 separate experiments.

(C) 1x10^{4} MACS-purified naïve CL4 CD8⁺ T cells were primed with plate-bound anti-CD3 mAbs (10 µg/ml) alone, or in a combination with either anti-CD28 mAbs (5 µg/ml) or plate-bound or soluble rICAM-1 (3 µg/ml). A proportion of CL4 T cells were pre-treated with rICAM-1 for an hour before the unbound ICAM-1 antibodies were washed, for 48 and 72 hours. Plates were pulsed with 1 µCi ^{3}H-thymidine in the last 8 hours. Bar charts show counts per minute (cpm). Error bars are mean ±SEM. Data are representative of 4 separate experiments. Statistical analyses were carried out using one way ANOVA followed by Bonferroni multiple comparison test. ** P< 0.01,* P< 0.05.

(D) MACS-purified CL4 CD8⁺ T cells were co-cultured for 48 and 72 hours with irradiated Renca-HA, Renca-HA/vector and various Renca-HA/ICAM-1 cells expressing relatively high, intermediate & low levels of ICAM-1. During the last 8 hours of each culture cells were pulsed with 1 µCi of ^{3}H-thymidine. Bar charts show counts per minute (cpm), error bars show mean ±SEM. and all data are representative of 2 separate experiments.

(E) Renca-HA/vector and Renca-HA/ICAM-1 cells that express high, intermediate and low levels of ICAM-1 were harvested and stained with live/dead aqua, HA-biotin (Bio) followed by SA-PE mAbs (left column) and anti-CD54-FITC mAbs (right column). Red filled histograms show the isotype control, whereas the blue histograms represent the HA-stained or ICAM-1-stained cells.

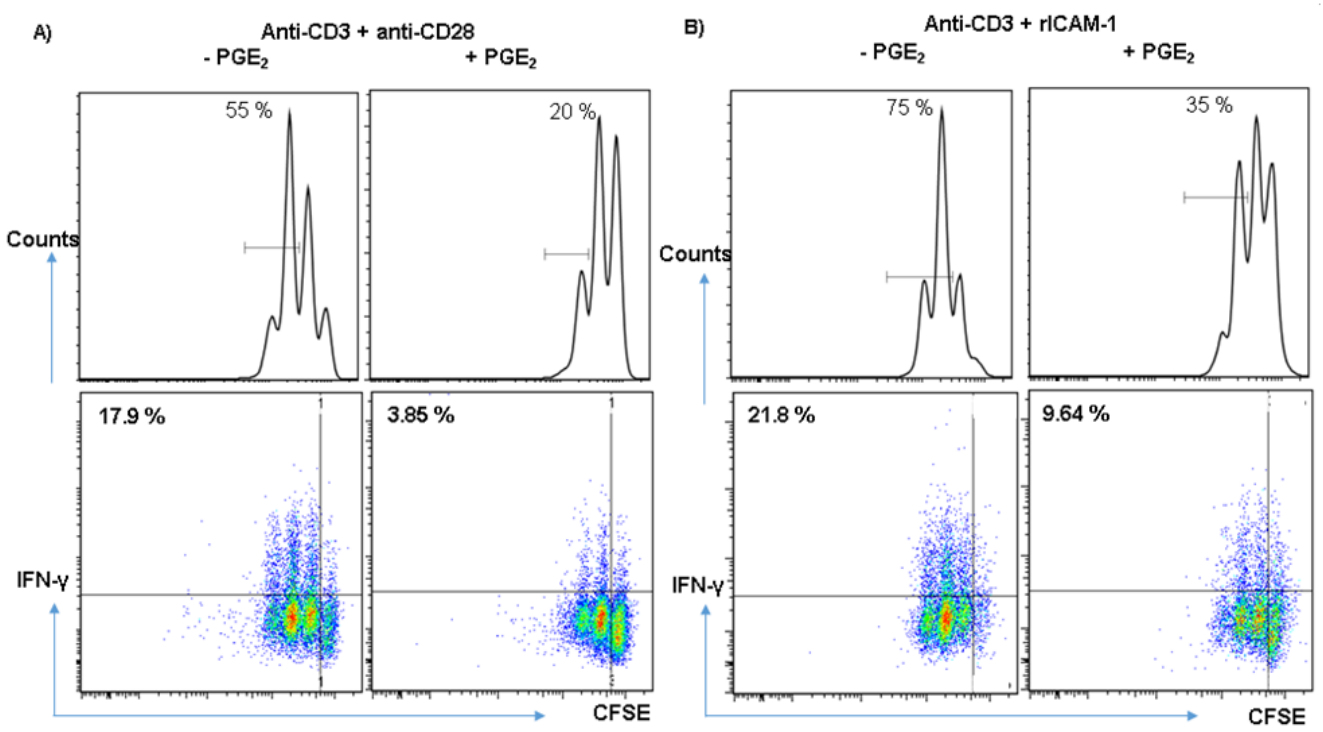

Figure 4: Proliferation and IFN-γ production by CL4 CD8⁺ T cells in the presence or absence of PGE_2.
1x10⁶ CFSE-labelled MACS-purified naïve CL4 CD8⁺ T cells were cultured with anti-CD3 mAbs (10 μg/ml), plus either anti-CD28 mAbs (5 μg/ml) **(A),** or plate-bound rICAM-1 (3 μg/ml) **(B)** in the presence or the absence of 1 μM PGE_2 for 48 hours. CL4 T cells were collected from the culture and re-stimulated with 1 μg/ml of both K^dHA and Golgi plug and incubated for a further four hours, and then stained with live/dead aqua dye before being permeabilized and stained intracellularly with anti-IFN-γ-APC mAbs. All Samples were analysed by flow cytometer. Dot plots are gated on live CFSE-labelled cells. FACS plots show the expression of CFSE *versus*; counts (top rows), or the expression of IFN-γ (bottom rows). Numbers in the top left corner of each plot show the percentage of positive proliferated cells for IFN-γ. Data are representative of 4 separate experiments.

It has been shown that some molecules such as matrix metalloproteinase 9 (MMP-9) can cause ICAM-1 to be shed from the surface of tumour cells and is involved in tumour evasion from the immune surveillance. ICAM-1 provides a docking site for pro-MMP-9 which proteolytically cleaves the extracellular domain of ICAM-1 leading to its release from the cell surface[16]. To determine whether or not PGE_2 directly affect the priming of naïve CL4 cells by causing ICAM-1 to be shed from the surface of tumour cells, Renca-HA cells were cultured in the presence of 1μM PGE_2 for 72 hours then stained for HA and ICAM-1 expression. Data presented in Figure 5A show that Renca-HA cells maintained the same levels of both HA and ICAM-1 following PGE_2 treatment suggesting that PGE_2 does not cause modulation or shedding of these molecules from the cell surface.

IFN-γ is able to exert its anti-tumour effects by directly acting upon the tumour cells themselves[17]; as evidenced by the fact that tumour cells lacking IFN-γR expression grow in mice despite the presence of effective anti-tumour immune responses, which would otherwise kill IFN-γR-sufficient tumour cells[18]. One mechanism through which IFN-γ is thought to reduce the growth of tumour cells *in vivo* is by enhancing tumour cell immunogenicity[19]. Indeed, IFN-γ has been shown to increase antigen presentation to CD8⁺ T cells by increasing MHC class I and ICAM-1 expression by tumour cells[3]. Similarly, IFN-γ treatment of Renca-HA cells also resulted in a marked increase in ICAM-1 expression (Figure 5B).

To determine whether or not the up-regulation of ICAM-1 is instrumental in abrogating the effect of PGE_2, we generated an ICAM-1 overexpressing Renca-T3 cell line (Renca-T3/ICAM-1), for co-culture with CFSE-labeled naïve CL4 T cells *in vitro*. The data show that co-culture with this cell line resulted in a significant increase in proliferation of CFSE-labelled naïve CL4 T cells, whereby greater than 65% of cells undergo at least three rounds of divisions compared with around 18% of CL4 T cells co-cultured in the presence of either un-transfected Renca-T3 or Renca-T3 empty-vector control cells (Figure 5C; top row). Moreover, co-culture with Renca-T3/ICAM-1 also resulted in a large increase in CTL effector function as evidence by the fact that around 60% of CL4 cells expressed IFN-γ, compared with only 18% of CL4 cells that were co-cultured in the presence of control Renca-T3 or Renca-T3 empty-vector cells (Figure 5C; bottom row). Taken together, these

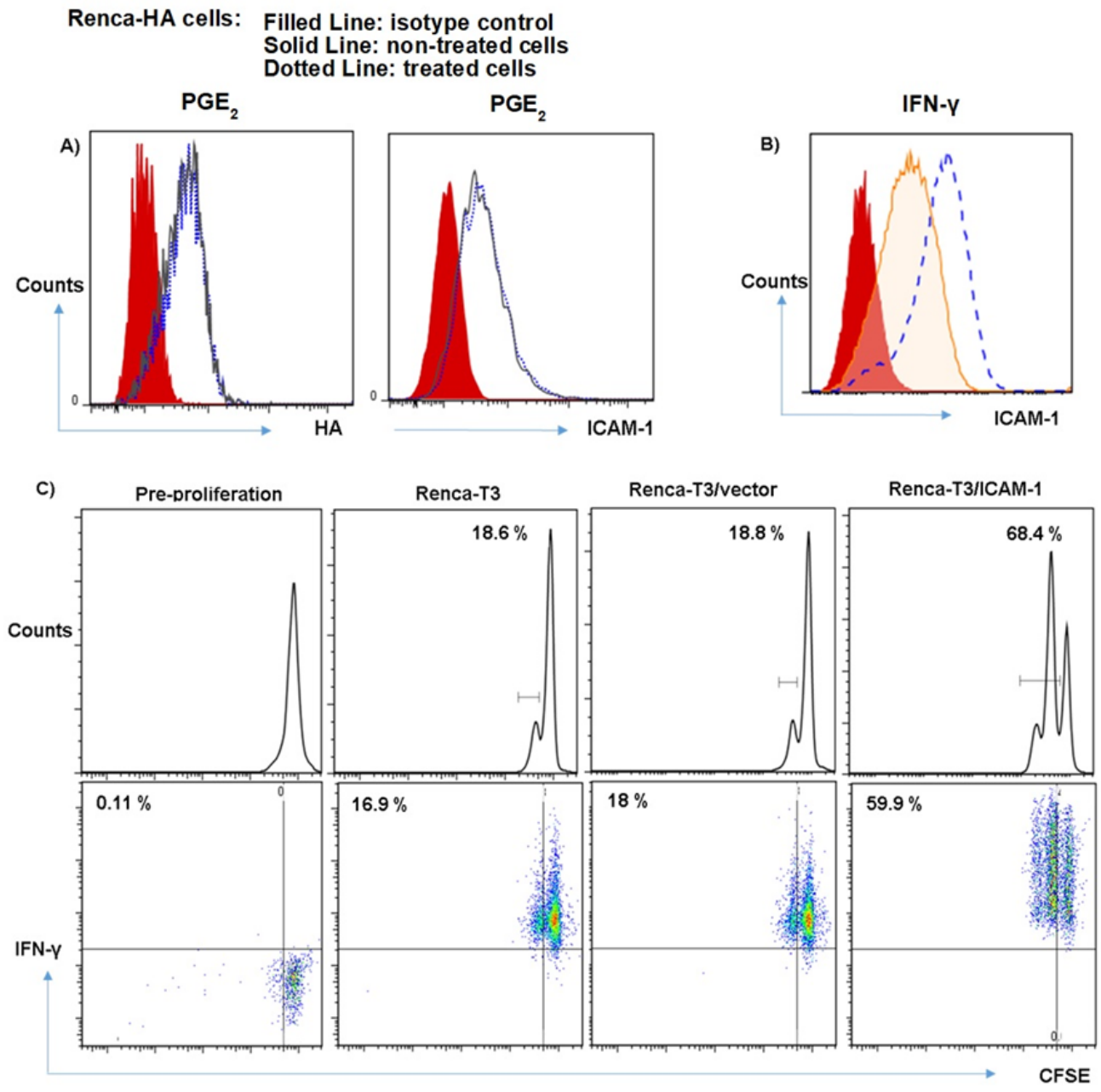

Figure 5: The correlation between IFN-γ, ICAM-1 and PGE_2 in CL4 $CD8^+$ T cell response

A) Renca-HA cells were treated with 1 μM exogenous PGE_2 for 72 hours. Renca tumour cells were collected and stained with live/dead aqua, HA-bio followed by SA-PE mAbs and anti-CD54-FITC mAbs. Filled red histograms show the isotype control, whereas the dotted blue lines represent PGE_2-non-treated culture and grey line histograms show PGE_2-treated culture. Data are representative of 4 separate experiments.

B) Renca-HA cells were cultured in the presence or absence of 10 ng/ml IFN-γ for 48 hours. Renca cells were collected and stained with anti-CD54-FITC mAbs and isotype control. Filled lines represent isotype control, whereas solid lines show IFN-γ-non-treated culture, and dashed lines show IFN-γ- treated cells.

C) CFSE-labelled MACS-purified naïve CL4 $CD8^+$ T cells were co-cultured with irradiated Renca-T3 and Renca-T3/vector and Renca-T3/ICAM-1 cells for 48 hours. CL4 T cells were collected and re-stimulated with 1 μg/ml of K^dHA and Golgi plug. CL4 T cells were then stained with live/dead aqua before being permeabilized and stained with anti-IFN-γ-APC mAbs. FACS plots show the expression of CFSE *versus* the production of IFN-γ (bottom row), and counts (top row). Numbers in each FACS plot show the percentage of proliferated CL4 T cells that are IFN-γ-positives (top left corner). Data are representative of 3 separate experiments.

data clearly demonstrate that overexpression of ICAM-1 by RencaT3/ICAM-1 cells is able to circumvent the PGE_2-mediated suppression of tumour-specific CTL responses.

Discussion

The requirement of ICAM-1-mediated co-stimulation in T cell priming has remained controversial. Some

reports suggest that ICAM-1 is not sufficient for $CD4^+$ T cell activation. In contrast, other reports propose the essential need of ICAM-1 for T cell priming but not for cytokine secretion, whereas others suggest that ICAM-1 can stimulate both activation and cytokine secretion[20]. However, studies using ICAM-1 knockout mice suggests that ICAM-1 is not essential in T cell-mediated tumour rejection if sufficient numbers of T cells are transferred[21]. In our study, Renca-HA tumour cells, that lack expression of both CD80/CD86, provide K^dHA-specific CL4 $CD8^+$ T cells with sufficient co-stimulation through ICAM-1 interactions with T cell-expressed LFA-1 that not only results in proliferation but also in IFN-γ production. This was evidenced by the clear finding that the presence of both anti-ICAM-1 and anti-LFA-1 mAbs during priming significantly reduces CL4 T cell proliferation and IFN-γ production[3]. Although the interaction of LFA-1 on T cells with soluble or plate-bound rICAM-1 can prime naive $CD8^+$ T cells, only plate-bound rICAM-1 enhances markedly the CL4 T cell response. This finding is consistent with a study in which T cells from mice lacking full-length cell-surface expression of either cell surface ICAM-1 or LFA-1 had significantly impaired T cell function[22]. It also supports the findings from another study in which over-expression of soluble rICAM-1 blocked LFA-1 on T cells preventing subsequent T cell activation due to the inability of LFA-1 to bind cell-surface ICAM-1. We have shown that overexpressing cell surface ICAM-1 on Renca-HA cells increased CL4 T cell proliferation and IFN-γ. This finding correlates with other studies in which the up-regulation of ICAM-1 on melanoma cells induced by retinoic acid, or ICAM-1 gene transfection, was found to improve the susceptibility of melanoma cells to lysis by lymphokine activated killer cells (LAK)[23]. Nevertheless, Renca-HA tumour cells continue to grow *in vivo* when they are injected into BALB/c mice either alone, or with large numbers of naïve CL4 cells. (CN Janicki and DJ Morgan; unpublished data). Following adoptive transfer, CL4 T cells undergo productive activation within the TDLNs, but they lose their effector function once they reach to the tumour site due to the immunosuppression created within the tumour microenvironment[24]. Many solid tumours create an immunosuppressive microenvironment through various immune escape strategies such as the production of PGE_2. Over-expression of COX-2 not only results in high levels of PGE_2 production, but also tumour cell metastasis by Renca-T3 cells and suppression of activation of naive CL4 $CD8^+$ T cells. Such abortive activation is evidenced by lack of proliferation and the absence of IFN-γ production due to the maintenance of low level ICAM-1 expression[12]. Whereas, exposure to IFN-γ was shown to markedly up-regulate ICAM-1. These findings correlate with other studies, which suggest that IFN-γ enhances antigen presentation by tumour cells through the increase of MHC class I expression[18]. We showed that disabling PGE_2 production by COX-2 inhibitor has shown to restore IFN-γ production by effector T cells[25]. Significantly, over expression of ICAM-1 on Renca-T3 cells restores CL4 T cell proliferation as well as IFN-γ production even in the PGE_2-rich microenvironment. Our findings clearly show that PGE_2 is able to inhibit both the direct priming of naïve tumour-specific $CD8^+$ T cells, as well as the effector function amongst a tumour-specific CTL, this effect is temporary and may be mitigated by increasing ICAM-1 expression on tumour cells which therefore enable the ligation of LFA-1 on CL4 with ICAM-1 on tumour cell which then allows the formation of a stable synapse between the two cell types and prolongs this interaction. Along with LFA-1/ICAM-1 binding, the interactions of TcR with K^dHA further increase Ca^{2+} influx and maintain LFA-1 in a high affinity state. In this state, the inhibitory effects, induced by PGE_2-mediated up-regulation of cAMP levels, on Ca^{2+} influx within CL4 T cells fail to override the stimulatory signals through stable LFA-1/ICAM-1 and TcR/K^dHA interactions. As a result, tumour-specific $CD8^+$ T cells are forced to proliferate more and produce high levels of IFN-γ and therefore may have greater potential to prevent tumour growth *in vivo*. These findings clearly indicate that drugs which can increase the cell-surface expression of ICAM-1 by tumor cells could provide us with a powerful immune-therapeutic tool to counteract the immunosuppressive action of tumor-derived PGE_2 to greatly enhance anti-tumor CTL responses, which may ultimately control tumor growth.

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