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Antiapoptotic seminal vesicle protein IV inhibits cell-mediated immunity

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Abstract

The in vitro effect of the seminal vesicle protein IV (SV-IV) on the cytotoxic activity of human natural or acquired cellular immunity has been investigated by standard immunological procedures, a ^{51}Cr-release cytotoxicity assay, and labeled-ligand binding experiments. The data obtained demonstrate that: 1) fluoresceinated or [^{125}I]labeled SV-IV binds specifically to the surface of human purified non-adherent monuclear cells (NA-MNC); 2) SV-IV suppresses the cytotoxicity of Natural Killer (NK) against K562 target cells, that of IL-2-stimulated NK (LAK) against DAUDI target cells, and that of VEL antigen-sensitized CTLs against VEL target cells; 3) treatment of K562 target cells alone with SV-IV decreases their susceptibility to NK-induced lysis. These findings, by indicating that the protein SV-IV has marked in vitro inhibitory effect on NK, LAK and CTL cytotoxicity, provide a better understanding of its immune regulatory functions.

Keywords: Protein SV-IV; NK; LAK; CTL; Immunomodulation; Binding experiments

1. Introduction

SV-IV (seminal vesicle protein no. 4, according to its mobility in SDS-PAGE) is a small (M_r = 9,758), basic (pI = 8.9), highly thermostable, secretory protein synthesized in large amount by the rat seminal vesicle (SV) epithelium under strict androgen transcriptional control (Ostrowski et al., 1979). Proteins immunorelated to SV-IV have also been found in several rat tissues (uterus, lung, liver, brain, etc.) and in human seminal fluid and SV secretion (Abrescia et al., 1985; Metafora et al., 1987a; Metafora et al., 1987b and unpublished results). SV-IV gene has been isolated, sequenced, cloned, and expressed in *E. coli* (D'Ambrosio et al., 1993). The mature protein, purified to homogeneity, has been extensively characterized (Ostrowski et al., 1979; Pan and Li, 1982; Kandala et al., 1983; Stiuso et al., 1999; Caporale et al., 2004). SV-IV is a highly flexible molecule whose biologically active configuration is monomeric, whereas its inactive one is trimeric (Stiuso et al., 1999; Caporale et al., 2004). The protein is biologically active between 2 and 30 μM. Below the latter value the monomeric active population exceeds the inactive trimeric one with a maximum at about 10 μM, while the opposite is true above this concentration (Stiuso et al., 1999; Caporale et al., 2004).

SV-IV possesses a nonspecies-specific ability to modulate inflammation and humoral and cell-mediated immune response (Metafora et al., 1989; Peluso et al., 1994; Romano-Carratelli et al., 1995; Tufano et al., 1996; Santagada et al., 2002), to stimulate horseradish and Se-dependent peroxidases (Metafora et al., 2001 and unpublished data), to protect its target cells against oxidative stress-associated apoptosis and, in particular, to defend nonself epididymal spermatozoa from immunological or reactive oxygen species (ROS) attacks in the female genital tract (Paonessa et al., 1984; Morelli et al., 2007). The anti-inflammatory properties are related to phospholipase A_2 (PLA_2) inhibition (Metafora et al., 1989), whereas the pro-inflammatory features are based on SV-IV ability to stimulate mast-cell and basophil degranulation with concurrent release of histamine and other mediators (Metafora et al., manuscript in preparation). The modulation of immune response is

caused by interference of the protein with macrophage-T cell cooperation (Metafora et al., 1989; Peluso et al., 1994; Romano-Carratelli et al., 1995; Tufano et al., 1996; Santagada et al., 2002).

Natural killer cells (NKs) and antigen-specific cytotoxic T lymphocytes (CTLs) are involved in host resistance to infections, immune surveillance against tumors, and rejection of transplanted tissues. NKs express on their surface specific markers (CD56 and CD16) and killer inhibitory receptors (KIR) (Yawata et al., 2006). These cells, in contrast with CTLs, are not MHC-restricted, even though their cytotoxic mechanisms are similar. Resting NKs express IL-2 receptors and respond to IL-2 acquiring enhanced cytotoxic function, recognizing a broader range of target cells (Moretta and Moretta, 2004) and taking the name of LAKs (lymphokine-activated killer cells).

In mammals, insemination transmits into female reproductive tract a variety of seminal factors that promote sperm survival, condition the female immune response to tolerate the conceptus, and induce molecular and cellular endometrial changes to facilitate embryo development and implantation (Robertson, 2005). In particular, it is worth noting that in rat semen is present, in free and sperm-bound form, the antiapoptotic and immunomodulatory protein SV-IV, whereas SV-IV immunorelated proteins have been detected in human seminal plasma (Ostrowski et al., 1979; Paonessa et al., 1984; Abrescia et al., 1985; Metafora et al., 1987b; Manco et al., 1988).

On the basis of these data and considerations, experiments were planned to verify whether SV-IV is able to exert its immunosuppressive activity on three models of human cell-mediated immune response not yet explored in detail by our group: a) the NK activity; b) the development and cytotoxic activity of LAK cells; c) the generation and cytotoxic activity of CTLs.

2. Materials and methods

2.1. Purification of protein SV-IV

The protein was purified to homogeneity from adult rat (Fisher-Wistar strain) seminal vesicle secretion according to a published procedure (Stiuso et al., 1999). A peculiar feature of SV-IV is the high thermoresistance: incubation in boiling water for 30-60 min neither precipitates the protein nor

changes its biological properties. The concentration of purified SV-IV was evaluated by its molar absorption at 276 nm (4100 M^{-1} cm^{-1}) (Stiuso et al., 1999; Caporale et al., 2004).

2.2. Preparation of fluoresceinated SV-IV (SV-IV_{FITC}) and its binding to human non-adherent mononuclear cells (NA-MNC)

SV-IV_{FITC} was prepared according to a conventional method (Goldman, 1968). Binding of SV-IV_{FITC} to NA-MNC (see below) was performed by mixing in the dark and at r.t. an appropriate number of cells suspended in PBS with an equal or 0.5 volume of 1.5 mg/ml (150 μM) SV-IV_{FITC} in PBS. Ten min later the cells were washed 3x with cold PBS and the binding of SV-IV_{FITC} to cells was monitored with a Leitz Aristoplan light fluorescence microscope equipped with a suitable filter system for FITC fluorescence, a x40 objective, and a Leica camera.

2.3. Cell lines

The NK- and LAK-susceptible human erythroleukemic MHC I^- cell line K562, the HLA class I deficient Burkitt lymphoma-derived cell line DAUDI, and the B-lymphoblastoid cell line VEL [transformed by Epstein–Barr virus (EBV) and kindly supplied by Dr. Stefano Vella, Istituto Superiore di Sanità, Rome, Italy] were cultured in suspension in RPMI-1640 medium (Hyclone Europe, Cramlington, UK), supplemented with 10% fetal calf serum (FCS; Hyclone Laboratories, Logan, UT), 2 mM L-glutamine, and antibiotics (Life Technologies Ltd., Paisley, Scotland), and hereafter referred to as complete medium (CM). The cell lines were maintained at 37°C in 5% CO_2 humidified atmosphere and subcultured three times weekly. All target cells used were > 90% viable, as measured by the Trypan Blue dye exclusion test and the carboxyfluorescein diacetate succinimidyl ester (CFSE) flow cytometry assay (Renovar, Inc., Madison, WI).

2.4. MNC, NK, LAK, and CTL preparation

Peripheral blood mononuclear cells (MNC), obtained from buffy-coats of healthy donors, were prepared by Ficoll-Hypaque density centrifugation (Lympholyte-H, Cedarlane Laboratories Limited, Hornby, Ontario, Canada). These experiments were approved by the Ethical Committee of the Blood Bank of the II Polyclinic Immuno-hematology Service (University Federico II, Naples, Italy). Adherent cells were removed by incubation at 37° C for 1 h. The remaining non-adherent cells (NA-MNC) were isolated by nylon wool columns. The NKs occurring in this fraction were purified by a MACS® two-step separation system (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany). The purified NKs were labeled with FITC-conjugated anti-NK antibodies, i.e. anti-CD16 and anti-CD56 (Becton Dickinson, Mountain View, CA). The efficiency of separation and the total $CD16^+CD56^+$ NK counting were assessed by a FACScan from Becton Dickinson. The yield was > 95% with a purity of 97±1%. The viability (see above) of NKs was > 95% in all the samples. The NK:T cells ratio in NA-MNC populations was evaluated by immuno-fluorescence flow cytometry, using specific cell surface markers for NKs ($CD3^-$, $CD16^+$, $CD56^+$) and T cells ($CD3^+$, $CD16^-$, $CD56^-$).

To generate LAKs, NA-MNC were treated with recombinant human IL-2 (Roche, Basel, Switzerland), alone or in combination with SV-IV. To evaluate the effect of SV-IV on mature LAKs, these cells untreated or treated for 2 h with SV-IV were used as effector cells (called hereafter mature LAKs or mature $LAK_{SV\text{-}IV}$, respectively) in cytotoxicity assays. Incubation of mature LAKs with SV-IV for 4 or 16 h did not change the results. The target cells used in LAK experiments were always ^{51}Cr-labeled DAUDI cells.

Allosensitized CTLs were generated by co-culturing for 5 days at 37°C MNC in presence of SV-IV with mitomycin C-inactivated (80 μg/ml) VEL cells at a MNC:VEL ratio of 40:1.

Cell number and viability of MNC were evaluated at the beginning and at the end of SV-IV treatment. After 2 or 120 h incubation, cell viability (see above) was higher than 97 ± 1.9 % in both control and SV-IV-treated cells.

2.5. ^{51}Cr-release cytotoxicity assay

The cytotoxic activity of NKs, LAKs, and CTLs was determined using a ^{51}Cr-release assay. Effector cells in 0.1 ml CM were plated in quadruplicate in U-bottomed 96-well microtiter plates (Greiner, Nurtingen, FRG) by making two-fold serial dilutions. Labeled target cells (2 x 10^4 in 0.1 ml) were added to give a final volume of 0.2 ml and an effector (E) to target (T) ratio ranging from 50:1 to 6.25:1. The plates were then centrifuged, incubated for 4 h at 37°C, recentrifuged, and 0.1 ml of supernatant from each well was counted by a gamma scintillation spectrometer (LKB, Sweden).

The percentage of specific lysis was calculated as follows:

$$\% \text{ Specific lysis} = \frac{\text{Test cpm} - \text{Autologous cpm}}{\text{Total cpm}} \times 100$$

where Test cpm is the mean cpm of ^{51}Cr released from labeled target cells in the presence of effector cells, Autologous cpm is the mean cpm of ^{51}Cr released from labeled target cells incubated with unlabeled autologous cells in place of effector cells, and Total cpm is the mean cpm corresponding to the total amount of ^{51}Cr incorporated into the total of labeled target cells.

2.6. Conjugation assay

The percentage of target cells with bound lymphocytes was determined at two effector:target ratios, 50:1 and 25:1. The purified NKs were preincubated with appropriate concentrations of SV-IV for 2 h. Following the incubation period, the SV-IV-containing solution was removed by centrifugation and replaced with CM. K562 target cells were then incubated with a suitable number of control or SV-IV-treated NKs in U-bottomed 96-well microtiter plates (2 x 10^4 tumor cells/well). Each experimental condition was tested in triplicate. The cells were incubated at 37° C, for 15 min, and then chilled on ice. Following incubation, the cells were gently resuspended with a micropipet, placed into a hemocytometer, and analyzed under a light microscope. The number of target cells with two or more lymphocytes bound to their surface and the total number of target cells were counted to

determine the percentage of tumor cells with bound lymphocytes. The minimum number of targets counted per determination was 100. The results of the SV-IV effect on the formation of clusters of fluorochrome-tagged NKs with K562 target cells obtained in a flow cytometry assay did not differ from the more simple optical method above described.

2.7. SV-IV negative controls used in the experiments

To rule out possible non-specific protein effects, we incorporated in our experimental design, where required, boiled and trypsinized SV-IV samples (2-30 μM) as negative controls. Boiling was used to inactivate residual trypsin activity in the digested samples. The immunosuppressive activity of SV-IV was totally abolished by the trypsinization. Simply heat-treated SV-IV was not used as negative control because the heat treatment (100°C for 30-60 min) alone was unable to biologically inactivate the thermostable protein (see also "Purification of protein SV-IV" in this section).

2.8. [^{125}I]SV-IV binding to purified human NA-MNC target cells

The SV-IV binding mixture contained in a final volume of 0.3 ml: 10^7 cells (NA-MNC), 140 mM NaCl, 50 mM Tris-HCl (pH 7.5), and 1.4 μg of [^{125}I]SV-IV ($5x10^6$ cpm) prepared by the chloramine T method (Hunter et al., 1978). After incubation, either at 37°C for 2 h or at 4° C for 30 min the assay mixtures were centrifuged (12,000xg at 4°C for 30 s) and the sedimented cells washed with RPMI 1640 until no radioactivity was detectable in supernatant. The washed cells were transferred before lysis in another polypropylene test tube to avoid contamination of final cell lysate with radioactivity not bound to the cells but adsorbed to the assay test tube walls. Cell lysis was carried out by suspending each cell pellet in 60 μl of double distilled water. After addition of 30 μl of a solution containing 15% β-mercaptoethanol, 190 mM Tris-HCl buffer (pH 6.8), and glycerol, the samples were boiled for 2 min and then analyzed by TCA-precipitable radioactivity counting and SDS-15% PAGE (Metafora et al., 1987a).

2.10. Statistical analysis

Differences in cytolytic effects produced by NK, LAK, and CTL effectors under various experimental conditions were evaluated by taking into account the % specific cytotoxicity at all E:T ratios. Therefore, P values were calculated using co-variance analysis performed on the regression of the percentage of specific ^{51}Cr release over the log of the number of effector cells.

Binding and cytotoxicity assay data were expressed as the mean ± standard error of the mean (SEM) obtained from 3 separate experiments. Each binding experiment included 9 samples; each sample was performed in triplicate. In cytotoxicity assays each point was carried out in quadruplicate. One-way analysis of variance (ANOVA) was used to assess statistical significance between means. Differences between means were considered significant when $P < 0.05$ using the Bonferroni post-test. All statistical analyses were performed with the GraphPad Instant software (Intuitive Software for Science, San Diego, CA).

3. Results

3.1. Inhibitory activity of SV-IV on NK cytotoxicity against K562 target cells

To evaluate the effect of SV-IV on NK function, purified NKs were exposed to various concentrations of SV-IV for 16 h in CM before the ^{51}Cr release assay, in which SV-IV-untreated ^{51}Cr-labeled K562 cells were used as target. The result of a representative experiment, concerning one out of five purified NK samples obtained from five different donors, is reported in Fig. 1A. The treatment with SV-IV shows a significant ($P<0.05$) and concentration-dependent inhibition of NK cytotoxic activity. Cell number (2×10^4 target cells) and viability (about 96%), assessed at the beginning and at the end of the incubation period with SV-IV, did not vary significantly among the various experimental conditions used.

Similar suppressive effect ($P<0.05$) on NK activity was found when K562 target cells alone were treated with 10 or 25 μM SV-IV for the same length of time (16 h) (Fig. 1B). Results did not differ when Raji cells were used as target cells in place of K562 (data not shown).

To investigate the effect of SV-IV on effector/target cell interaction, the 4 h cytotoxicity assay was performed by treating the effector/target mixture with 10 μM SV-IV. The data from a representative experiment reported in Fig. 1C show that the percentage of lysis of K562 target cells produced by intact purified NKs was significantly ($P<0.05$) reduced by SV-IV.

Additional experiments were performed to evaluate the effect of SV-IV on the conjugation process between purified NKs and K562 target cells. The results demonstrated that 10 μM SV-IV inhibited ($P<0.05$) by about 48% (mean value) the NK binding to K562.

3.2. The functional activity of IL-2-stimulated NKs is suppressed by SV-IV

To evaluate the effect of SV-IV on the generation and lytic function of IL-2-activated NKs, NA-MNC were exposed for 2, 4 or 16 h to 5 μM SV-IV either before or after 4 days of culture with IL-2 and then tested for cytotoxic activity against ^{51}Cr-labeled DAUDI target cells. The result of a representative experiment (Fig. 1D) demonstrates that SV-IV does not affect ($P<0.05$) the LAK generation. In contrast, the cytotoxic activity of cells treated with SV-IV for 16 h after IL-2 stimulation (LAK_{SV-IV}) was significantly ($P<0.05$) inhibited (Fig. 1E). Similar results were obtained when LAKs were treated with SV-IV for 2 or 4 h.

3.3. The alloantigen-induced generation of CTLs is decreased by SV-IV

To assess the effect of SV-IV on CTL induction, NI-MNC (Non Immunized-MNC) were stimulated for 5 days (day 1-5) with mitomycin C-inactivated VEL target cells. Five μM SV-IV was added or not (controls) to both NI- and I (Immunized)-MNC cultures on day 1. On day 5, the effectors were harvested, washed, and evaluated for CTL activity against ^{51}Cr-labeled VEL cells. The results of a representative experiment concerning 5 samples from different donors (Fig. 1F) show that the protein produced a statistically significant ($P<0.05$) reduction of CTL activity.

3.4. SV-IV$_{FITC}$ and [^{125}I]SV-IV binding to the surface of human purified NA-MNC

The treatment of NA-MNC cells at r.t. with 15 μM SV-IV$_{FITC}$ generated a nonhomogeneous fluorescent coat on their surface (Fig. 2).These data are consistent with similar binding observations already published (Metafora et al., 1989; Morelli et al., 2007).

Native [^{125}I]SV-IV was incubated with NA-MNC to evaluate the number of specific SV-IV binding sites occurring on the surface of these cells. The low temperature used (4°C; see Materials and Methods) was chosen in order to avoid internalization of the protein during the binding assay. Scatchard analysis of radioactivity bound to cell surface either in presence or in absence of native or trypsinized unlabeled SV-IV, or other unlabeled proteins [BSA or proteins (histones, cyt-c) with a M_r and a pI similar to that of SV-IV], showed the specificity of this binding. The binding sites contained only saturable components displaceable in a dose-dependent manner only by unlabeled native or trypsinized SV-IV and not by unlabeled BSA, histones or cyt-c. The number of specific binding sites, calculated by Scatchard analysis, was $1 \pm 0.2 \times 10^5$/cell ($P<0.05$) with a Kd of $1 \pm 0.5 \times 10^{-8}$ M ($P<0.05$). These results fully confirmed previously published binding data of SV-IV to its target cells (Stiuso et al., 1999; Romano-Carratelli et al., 2002). The binding at 37°C was found about 25% lower, probably as consequence of a slight release of counts from cell surface caused by the relatively high Kd. Additional experiments proved that the labeled protein (FITC- or iodinated-SV-IV) remained structurally (SDS-PAGE) intact into medium up to 4 days incubation at 37°C. Other data indicate that the putative SV-IV receptors probably signal via a protein tyrosine kinase-operated pathway (Morelli et al., 2007).

4. Discussion

The results reported in this paper show that SV-IV inhibits in vitro the cytolytic effects of human NK, LAK, and antigen-activated CTL effectors, whereas the treatment of target cells alone with the protein decreases their susceptibility to NK-induced lysis. These data confirm the immunosuppressive activity of SV-IV previously observed in other experimental settings (Paonessa

et al., 1984; Metafora et al., 1989; Peluso et al., 1994; Romano-Carratelli et al., 1995; Romano-Carratelli et al., 2002).

The concentration of SV-IV used was in the 2-15 μM range, a value comparable to physiological levels. In fact, in the rat female reproductive tract following coitus the SV-IV concentration ranges between 12 and 48 μM, whereas the concentration in the aqueous extracts of other organs (liver, brain, lung, uterus, etc.) is lower, ranging from 2 to 10 μM (Stiuso et al., 1999). It is worth noting that at these concentrations SV-IV is mainly monomeric and its biological activity is maximal (Stiuso et al., 1999).

To exclude that the inhibition of cell-mediated immunity by SV-IV (see above) was due to generic toxicity, its effect on morphology, viability, and proliferation of resting and IL-2-stimulated NKs was analysed. In comparison to untreated samples, no changes in morphology and viability were observed in SV-IV-treated cells up to 2 h and 4-5 days of culture. Similar results were obtained with resting or antigen-stimulated CTLs. In addition, the possibility that the immunosuppression was due to aspecific cytotoxic properties can be ruled out by the following observations: 1) immunosuppression was detectable on activated MNC (see Results) only at low concentrations (5 to 30 μM), rich in biologically active SV-IV monomers; at similar low concentrations SV-IV did not inhibit but, in contrast, stimulated quiescent lymphocytes to synthesize and release specific cytokines (Tufano et al., 1996); 2) at concentrations higher than 30 μM, rich in biologically inactive SV-IV trimers, no cytotoxic effects were observed (Stiuso et al., 1999); 3) the inhibitory effects of SV-IV on proliferation were found only for immunocompetent cells (Metafora et al., 1989); 4) in addition, SV-IV has been recently demonstrated to possess intrinsic antiapoptotic activity (Morelli et al., 2007).

Our studies raise the possibility that SV-IV could be active in preventing lymphocyte cytotoxicity towards implanting embryos also in vivo. Although a direct evidence of this is missing, we have demonstrated that SV-IV-immunorelated proteins are present (standard competition RIA and SDS-15% PAGE) in rat uterus (uterine fluid, endometrium, and decidua lysates) after insemination

(Metafora et al., 1987a and unpublished results). In humans, however, data on the occurrence in uterus of SV-IV-immunorelated proteins are not available at present, even though we have found these type of proteins in human SV secretion and seminal fluid (Metafora et al., 1987b). Experiments are in progress to acquire further information on this point.

In our experimental design the possible protective effect of SV-IV against maternal cell-mediated immune response and ROS attacks on implanting hemi-allogeneic blastocysts has been evaluated in vitro by using human peripheral blood NKs rather than decidual NK cells: this is an imperfect model (as are all models), but the phenomenon described seems to be interesting regardless the source of the NKs tested .

Fig.1D shows that SV-IV does not influence LAK generation probably because the activation of NKs by IL-2 is either insensitive to SV-IV or, alternatively, is subjected to unknown control mechanisms during in vitro culture. This finding is consistent with the fact that IL-2 is poorly represented at fetal-maternal interface in the implantation site, where LAKs are few (King and Loke, 1990) and probably also immunosuppressed by uterine SV-IV (Stiuso et al., 1999), uteroglobin (Mukherjee et al., 2007), and some other factor (TGF β-1, IL-10, decidual T_{Reg} and γ/δT cells, etc.). This is in line with the fact that mature LAKs exposed to SV-IV on day 4 for only 2 h, immediately before the cytotoxicity assay, showed a reduction of their cytolytic potential (Fig. 1E).

The hemi-allogeneic mammal early embryo has to face at its uterine implanting site the maternal innate and acquired immune alloreactivity and possible ROS attacks. The blastocyst, however, is well equipped to counteract this harmful situation. Synthesis of HLA-G as a substitute of missing HLA class I, allows blastocysts not only to silence uNK and CTL anti-blastocyst cytotoxicity, but also to obtain uNK cooperation for embryo development by inducing these cells to secrete embryotrophic cytokines and factors promoting cell growth, angiogenesis, and EVT survival and invasiveness (Rouass-Freis et al., 1997; Moretta and Moretta, 2004; Perham, 2004; Rajagopalan et al., 2006). In addition, the occurrence in uterus at blastocyst implanting time of a high number of protective molecules (Leukemia Inhibitory Factor, heme oxygenase HO-1, IL-10, TGF-β, etc.),

tolerogenic $CD4^+CD25^+Foxp3^+$ T_{Reg} cells (Zenclussen et al., 2006), and mast cells (Dey et al., 2004), together with the recent finding in long–term allograft tolerance systems of a novel T_{Reg}-IL-9-mast cell immunosuppressive relationship [T_{Reg} (TGF-β) → IL-9 → mast cell → (TGF-β)] (Lu et al., 2006; Zenclussen et al., 2006;), is of substantial help for the formation of an immunotolerant and antiapoptotic implantation site. The presence of SV-IV in the uterus at this location following insemination could also contribute to the definition of the immunotolerant characteristics of the site, although the evidence that the protein is present in this location at the implantation time is only circumstantial (SV-IV is present in rat ejaculated semen in free and sperm-bound form; an SV-IV immunorelated protein has been found in rat uterine lysates and in human seminal plasma) (Ostrowski et al., 1979; Paonessa et al., 1984; Abrescia et al., 1985; Metafora et al., 1987a; Metafora et al., 1987b and unpublished results from our lab; Manco et al., 1988). In rats, this speculation seems supported by mixed cell culture experiments [maternal splenocytes (responder cells) co-cultured with mitomycin-C-inactivated hemi-allogeneic blastomeres, isolated from implanting blastocyst and treated or not with 5 μM SV-IV] performed in our laboratory and demonstrating that the immunogenicity of blastocyst blastomeres, as evaluated by the [^{3}H]thymidine incorporation assay, is suppressed (about 80%) in presence of SV-IV (SV-IV-untreated blastomeres: 12,800 cpm ± 400 cpm; SV-IV-treated blastomeres: 2350 ± 220 cpm; splenocyte only: 230 ± 75 cpm; splenocyte plus Con A: 31,000 ± 1200 cpm). These SV-IV-induced immunosuppressive findings are in line with the protective effect of SV-IV on Raji, K562, and Daudi target cells against the NK cytotoxicity (see Results). In this setting it appears also relevant: 1) the SV-IV ability to activate mast cell degranulation with release of immunosuppressive and antiapoptotic histamine and TGF-β (Hansson et al., 1999; Metafora et al., manuscript in preparation); 2) the SV-IV ability to protect mouse zygote growth up to blastocyst stage in the semi-solid Fell and Robison culture medium nonpermissive for fertilized egg early development (Morelli et al., 2007). 3) other data showing the critical role of oxidative stress in jeopardizing embryo survival during early development (Guerin et al., 2001).

In conclusion, the experimental results reported in this paper, when analysed in the context of other published biological features of SV-IV, not only provide a better understanding of its immune regulatory functions, but raise also the possibility of a contributory role (at least in rodents) in the multifactorial orchestration at the uterine implantation site of maternal immunotolerance and blastocyst survival. More convincing data on SV-IV presence at the uterine implantation site and specific in vivo experiments (e.g. null mutation of SV-IV gene) are, however, demanded to provide conclusive evidence for this role.

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LEGENDS TO FIGURES

Figure 1. Effect of SV-IV on the cytotoxic activity of purified NK cells using K562 as target cells: (A) inhibitory effect on SV-IV-treated NK cells; (B) inhibitory effect on SV-IV-treated K562 cells; (C) inhibitory effect on SV-IV-treated effector-target cell mixture. Inhibitory effect of SV-IV on LAK cell cytotoxicity: (D) during the LAK generation phase; (E) on mature LAKs. Inhibitory effect of SV-IV on CTL generation: (F) MNC were stimulated for 5 days (day 1-5) with mytomicin C-inactivated VEL cells. Five μM SV-IV was added to MNC cultures on day 1. On day 5, the VEL-sensitized CTL effector cells were harvested, washed, and evaluated for cytotoxic activity. Experimental details are in Material and Methods. Error bars indicate SEM.

Figure 2. Binding of fluoresceinated SV-IV to NA-MNC cell surface. Experimental details are in Material and Methods.

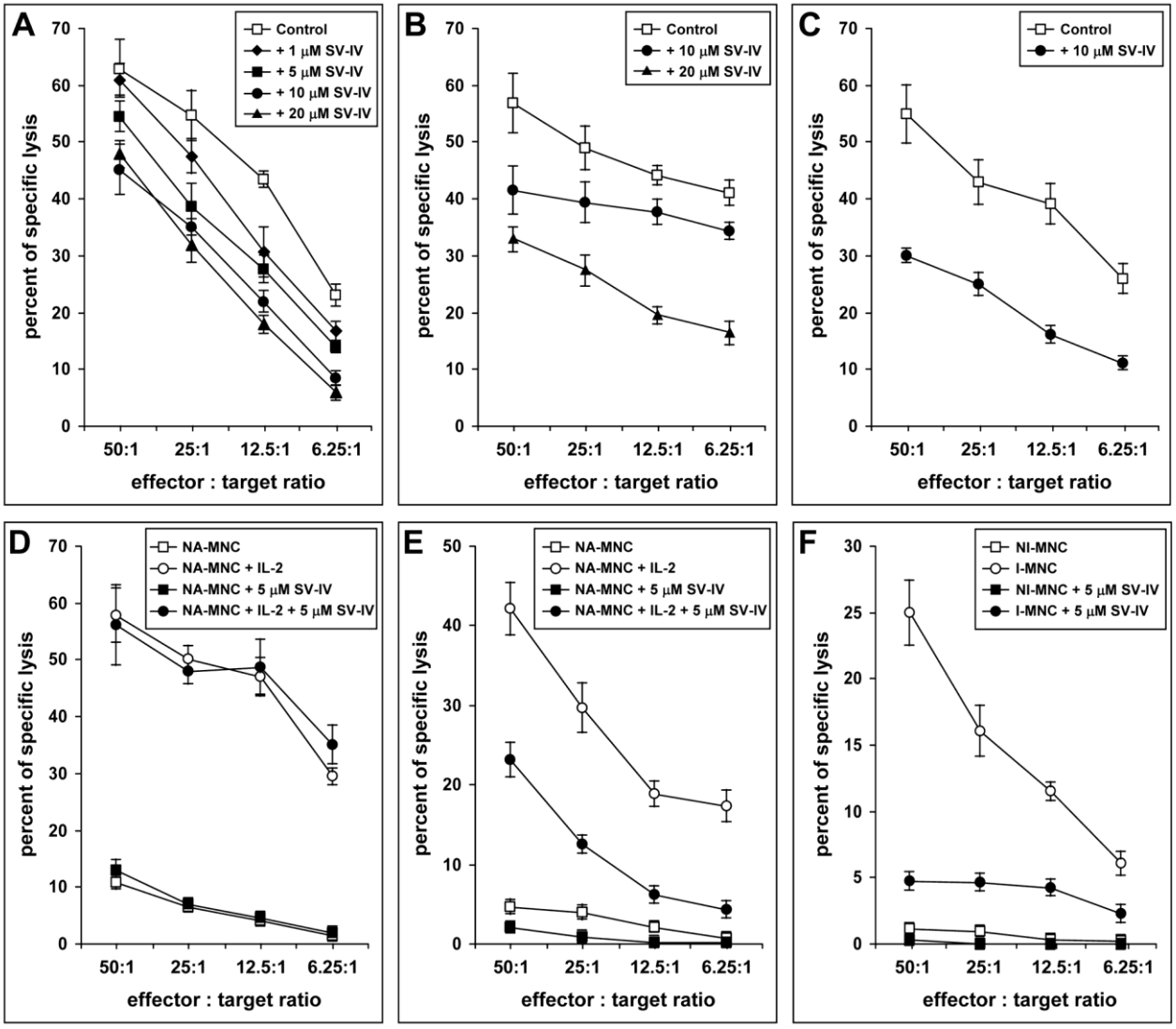
A
Control
+ 1 μM SV-IV
+ 5 μM SV-IV
+ 10 μM SV-IV
+ 20 μM SV-IV
percent of specific lysis
effector : target ratio
50:1
25:1
12.5:1
6.25:1
B
Control
+ 10 μM SV-IV
+ 20 μM SV-IV
C
Control
+ 10 μM SV-IV
D
NA-MNC
NA-MNC + IL-2
NA-MNC + 5 μM SV-IV
NA-MNC + IL-2 + 5 μM SV-IV
E
F
NI-MNC
I-MNC
NI-MNC + 5 μM SV-IV
I-MNC + 5 μM SV-IV

Figure 1

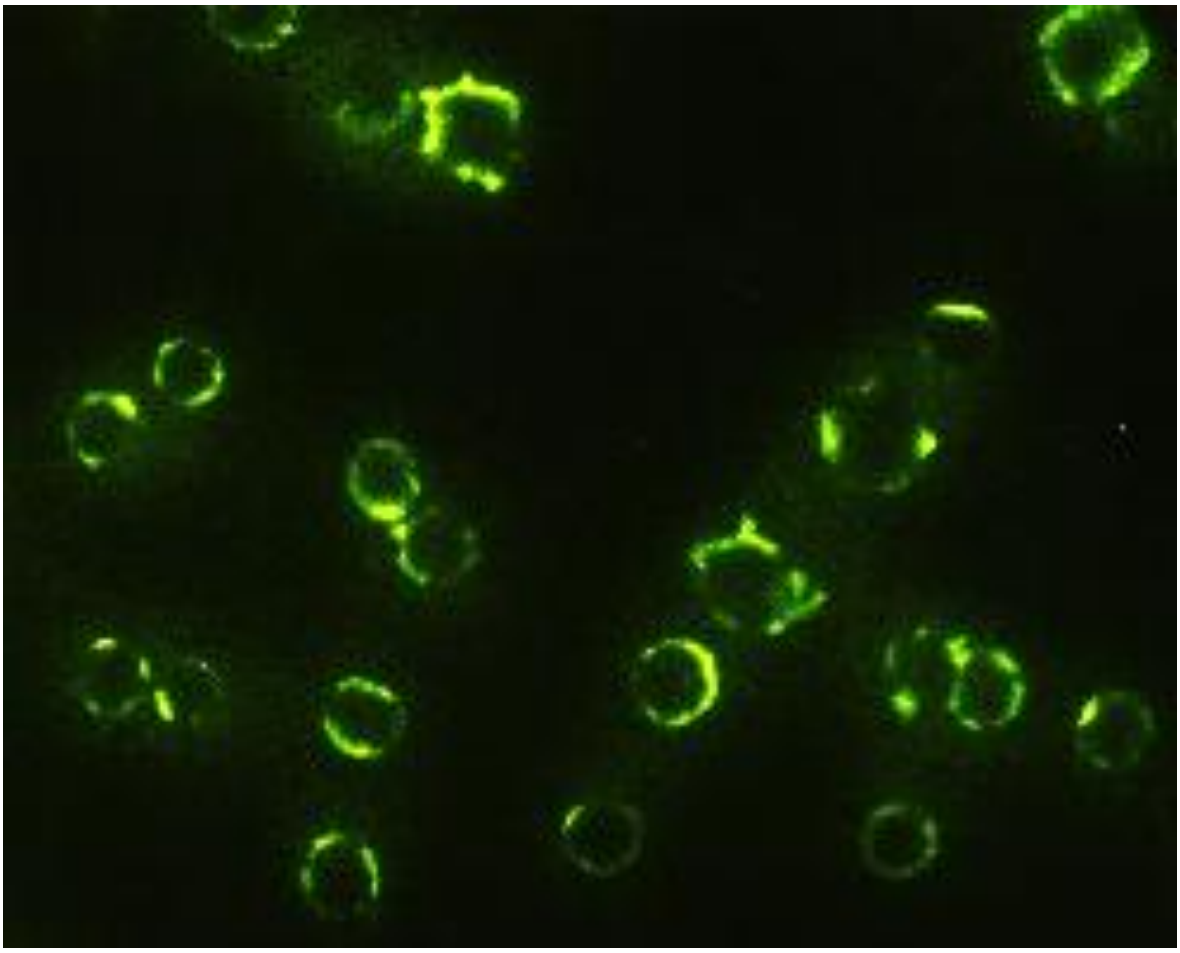

Figure 2

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Anti-apoptotic seminal vesicle protein IV inhibits cell-mediated immunity

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Abstract

The *in vitro* effect of seminal vesicle protein IV (SV-IV) on the cytotoxic activity of human natural or acquired cellular immunity has been investigated by standard immunological procedures, a 51Cr-release cytotoxicity assay, and labeled-ligand binding experiments. The data obtained demonstrate that: (1) fluoresceinated or [125I]-labeled SV-IV binds specifically to the surface of human purified non-adherent monuclear cells (NA-MNC); (2) SV-IV suppresses the cytotoxicity of natural killer (NK) cells against K562 target cells, that of IL-2-stimulated NK (LAK) cells against DAUDI target cells, and that of VEL antigen-sensitized cytotoxic T lymphocytes (CTLs) against VEL target cells; (3) treatment of K562 target cells alone with SV-IV decreases their susceptibility to NK-induced lysis. These findings indicate that the protein SV-IV has a marked *in vitro* inhibitory effect on NK, LAK and CTL cytotoxicity, providing a better understanding of its immune regulatory functions.

Keywords: Protein SV-IV, NK, LAK, CTL, Immunomodulation, Binding experiments

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