Med Microbiol Immunol (1999) 187: 191–198

ORIGINAL INVESTIGATION

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Induction of antibody responses in the common mucosal immune system by respiratory syncytical virus immunostimulating complexes

Received: 23 October 1998

Abstract Immunostimulating complexes (ISCOMs) containing envelope proteins of respiratory syncytial virus (RSV) were explored as a mucosal delivery system for the capacity of inducing a common mucosal antibody response. Two intranasal (i.n.) administrations of BALB/c mice with ISCOMs induced potent serum IgG, and strong IgA responses to RSV locally in the lungs and the upper respiratory, and remotely in the genital and the intestinal tracts. Virtually no measurable IgA response was found in these mucosal organs after two subcutaneous (s.c.) immunizations. Virus neutralizing (VN) antibodies were detected in serum and in all of the mucosal organ extracts after both s.c. and i.n. immunizations indicating that the neutralizing epitopes were preserved after both mucosal and parenteral modes of administration. While the mucosal IgA response appears to be of mucosal origin, the IgG antibodies to RSV detected in the mucosal organs were likely of serum origin. However, the mucosal VN antibodies correlated with the IgG rather than the IgA levels. An enhanced IgA response to gp120 in various mucosal organs was recorded after i.n. immunization with gp120 incorporated in RSV ISCOMs, indicating a role of RSV envelope proteins in enhancing and targeting mucosal responses to passenger antigens.

Key words Immunostimulating complexes · IgG · IgA · Virus neutralizing antibody · gp120

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Introduction

In spite of the fact that the majority of pathogens gain access to the host via mucosal surfaces, most vaccines of today are administered parenterally emphasizing a systemic response, providing an insufficient protection against mucosal infections. Therefore, there is a general need for vaccine delivery systems which induce potent immune responses in various mucosal sites which may not be readily accessible for administration. The best known adjuvants facilitating mucosal administration of non-replicating antigens are cholera toxin (CT) [27] and *Escherichia coli* heatlabile enterotoxin (LT) [9]. However, their practical value in the unmodified forms is limited because of toxic properties though both mutant CT [37] and LT [15] are showing promises for evasion of these problems.

The immunostimulating complex (ISCOM) is a potent mucosal and parenteral adjuvant and delivery vehicle, enhancing both system and mucosal immune responses [21]. Recently it has been shown that ISCOMs have different immunological properties from CT [30], which make them interesting alternatives to CT and LT. The ISCOM is a 40-nm particle in which antigens are arranged in a multimeric form together with the inbuilt *Quillaja* saponin adjuvant [19, 20]. The flexibility of ISCOMs allows virtually any kind of antigen to be incorporated, i.e., antigens from the natural micro-organisms, rDNA products or synthetic peptides [6, 17]. The adjuvant component can also be exchanged to alter the immunomodulating activity. Intranasal (i.n.) immunization with influenza virus ISCOMs induced a specific antibody response of all immunoglobulin classes and subclasses, and cellular immune responses in the lungs [13]. A number of studies have shown that ISCOMs containing influenza and HIV envelope proteins induce cytotoxic T lymphocytes (CTL) by i.n. route [13] and parenteral route [32], respectively. Ovalbumin (OVA) ISCOMs were also shown to be able to overcome tolerance after oral administration [23] and to induce potent mucosal and systemic immune responses by i.n. immunizations [7]. It has recently been shown that

OVA ISCOMs efficiently induce strong systemic and intestinal immune responses after oral administration, being independent of IFN-γ and IL-4 for induction of mucosal IgA antibody and cell-mediated delayed-type hypersensitivity (DTH) and CTL responses [30]. A study carried out by Claassen et al. [2] showed that ISCOMs containing the G protein of rabies virus were efficiently taken up by Peyer's patches through M cells in contrast to the whole virus particles.

An experimental RSV ISCOM vaccine was first introduced by Trudel et al demonstrating its effectiveness in inducing serum neutralizing antibodies [33] and CTL [34] responses in mice. Recently, we have shown in mice that two i.n. administrations of RSV ISCOMs induce potent and long-lasting IgA response in respiratory tract and IgG in serum with low dose (5 μg) of antigen. The serum antibody response was of similar magnitude as that induced by subcutaneous (s.c.) administration [11]. The objective of this study was to use RSV as a model to explore ISCOMs as a delivery system for induction of a common mucosal antibody response by i.n. administration, measured at the local inductive sites of the upper respiratory tract (URT) and lungs, and the remote sites of the intestinal and genital tracts. In view of the capacity of the RSV envelope proteins to induce IgA response in remote mucosal surfaces, they were also explored for mucosal targeting in the ISCOMs for a passenger antigen gp120 of HIV-1.

Materials and methods

Virus and cells

The Long strain of human RS virus (ATCC VR-26) kindly supplied by Dr. Claes Örvell (Huddinge University Hospital, Stockholm), was propagated on MA 104 cells (ECACC no. 85102918). Cells were grown in Full Dulbecco's Modified Eagle Medium (FDMEM, National Veterinary Institute, Uppsala, Sweden) supplemented with 100 μg/ml of kanamycin, 2 mM glutamine, and 10% fetal calf serum (GibcoBRL, Life Technologies AB, Täby, Sweden).

Preparation of RSV ISCOMs

The preparation procedure and biochemical characterization of ISCOMs were carried out essentially as previously described [16, 18, 33] with some minor modifications [11]. The ISOCMs contained mainly fusion (F) and to a lesser extent attachment (G) proteins of RSV with a Quil A to protein ratio of 10:1 on weight basis.

Preparation of gp120 and gp120/RSV ISCOMs

To incorporate of gp120 into ISCOMs, gp120 was lipidated essentially as described by Wilson et al. [35]. To prepare gp120 ISCOMs and gp120/RSV ISCOMs, 1 mg lipidated gp120 (together with 0.8 mg solubilized RSV glycoproteins for gp120/RSV ISCOMs) was added to 1.8 mg of each cholesterol and phosphatidyl choline in octylglucoside and 6.3 mg Iscoprep 7.0.3 (Iscotec AB, Uppsala, Sweden). The mixture was incubated for 2 h at 20 °C with agitation prior to dialysis against PBS at least for 48 h with five changes of buffer.

The two ISCOM preparations were analyzed by centrifugation through a 10–50% (w/w) sucrose density gradient at 200,000 g for 18 h at 10 °C. The incorporation of antigens into ISCOMs was verified by ELISA, Western blot and electron microscopy showing comigration of proteins and ISCOM structures.

Mice

Female BALB/c mice, 8–12 weeks of age, were obtained from the National Veterinary Institute, Uppsala, Sweden. The mice were screened for viral, bacterial and mycoplasma infections, and kept in accordance with the national guidelines.

Immunizations

Three groups of six BALB/c mice were immunized twice 6 weeks apart as follows: group 1, 1 μg RSV ISCOMs s.c.; group 2, 5 μg RSV ISCOMs i.n. (i.n.-5 μg); group 3, 10 μg RSV ISCOMs i.n. (i.n.-10 μg). A fourth group of six mice were kept as non-immunized controls.

Another two groups of 10 BALB/c mice were immunized twice 4 weeks apart. Group 1, 10 μg gp120 in gp120 ISCOMs i.n.; group 2, 10 μg gp120 and 10 μg RSV in gp120/RSV ISCOMs i.n., A third group of ten mice were kept as control.

For i.n. immunization, the ISCOMs were suspended in a volume of 20 μl PBS, while the s.c. dose was suspended in a volume of 200 μl PBS; i.n. immunizations were carried out under anesthesia with methoxyflurane administrated by inhalation.

In an introductory experiment, five groups of BALB/c mice were immunized with: 5 μg/mouse of RSV ISCOMs i.n.; 1 μg/mouse of RSV ISCOMs s.c.; 500 μl of inactivated RSV s.c. containing the same amount of F protein as in the ISCOMs preparation for s.c. administration and 40 μl of live RSV (i.n.) containing 10^6 $TCID_{50}$/ml, respectively.

Collection of samples and extraction of immunogloblins

Blood samples for serological evaluation were collected from the retroorbital plexus 2 weeks after the second immunization. Lungs, URT, and genital and intestinal tracts were taken at the same time for antibody extraction. Six mice from each group were killed and their serum and organ samples collected. Antibodies from the mucosal organs were extracted with 2% saponin as described by [1, 11]. The intestinal and genital tracts were treated in a similar way. To test the leakage of IgG antibodies from serum to the organs, a monoclonal antibody (mAB) to pseudorabies (PRV) was injected i.v. After 20 h, the antibody titers to PRV were measured in serum and organs by ELISA; 0.25%, 2.36%,5.49% and 6.76% of the PRV mAb levels found in the serum were detected in extracts from the lungs, intestinal, upper respiratory and genital tracts, respectively. This result is similar to that observed by Bergquist et al. [1], indicating that the direct IgG contamination from the blood is insignificant.

For samples to be assayed for virus neutralizing (VN) antibody, the saponin was exchanged with distilled water for the extraction of antibodies to avoid the lytic and cell toxic effects [12].

Quantification of antibodies and immunoglobulins

The IgA and IgG antibodies to RSV and the concentrations of total IgA and IgG in sera and in extracts were determined by ELISAs essentially as described previously [10, 11]. For quantification of total IgA and IgG concentrations, the plates were coated with affinity-purified goat anti-mouse IgA (α-chain specific, Sigma) and goat anti-mouse IgG (Fc specific, Sigma), respectively. Twofold serial dilutions in PBST with 5% non-fat dry milk of both test samples and standard solutions of RSV-specific IgG and IgA were prepared. In another series nonspecific IgG and IgA were applied to the ELISA plates, which were incubated at room temperature for 2 h, followed by incubation with biotin-labeled goat anti-mouse IgA (α-chain specific, Southern Biotechnology Associates, Birmingham, USA) or with biotin-labeled goat anti-mouse IgG (γ heavy chain specific,

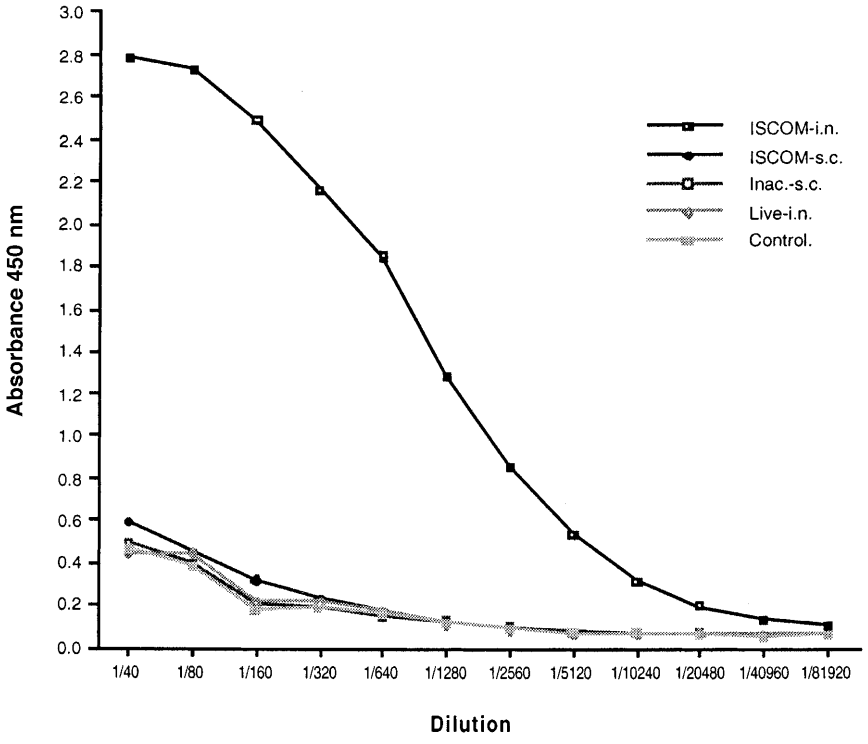

Fig. 1 The IgA response to RSV in lungs from mice measured by ELISA 2 weeks after two immunizations (6 weeks apart), i.n. with RSV ISCOMs (*ISCOM-i.n.*), s.c. with RSV ISCOMs (*ISCOM-s.c.*), s.c. with inactivated RSV(*Inac.-s.c.*), or i.n. with live RSV (*Live-i.n.*) and the control group (*Control*). Each group comprised six mice (*RSV* respiratory syncytical virus, *ISCOM* immunostimulating complex, *ELISA* enzyme-linked immunosorbent assay, *i.n.* intranasal, *s.c.* subcutaneous)

Southern Biotechnology Associates) and streptavidin-horseradish peroxidase (DAKO A/S, Denmark).

The IgG and IgA anti-RSV activities of the samples were expressed as U/ml or U/g based on the activities in a standard pool of mouse sera and a standard pool of lung extracts, for which end-point titration had been assigned to 26 kU/ml IgG and 1.3 kU/ml IgA antibodies to RSV. Purified mouse IgG from pooled normal mouse serum (Sigma) and monoclonal IgA (Sigma) were used as reference standards for concentrations of total IgG and IgA. The values were calculated from the linear parts of standard curves, and corrected for dilutions of the samples. Antibody levels were expressed as a proportion of the total concentrations of corresponding immunoglobulins to compensate for the variation in flow rate of secretion (known to inversely affect immunoglobulin concentrations) and for the dilution factor [29] in the case of secretions collected by extraction.

ELISA for antibodies against gp120 was carried out in a similar manner except that the plates were coated with 200 ng/well gp120.

Virus neutralization assay

The neutralizing activity was measured on pooled sera or pooled organ secretions by a microneutralization test, similar to the method used by Trudel et al. [33] with some modifications as detailed by Hu et al. [12]. Titers for virus neutralization test were expressed as the reciprocal of last dilution exhibiting 50% residual infectivity (ND_{50}/ml serum or tissue).

Statistical analysis

The Mann-Whitney U two-tailed test was used to compare differences of significance between the groups, and simple linear regression as well as Pearson's correlation coefficient were used for the relationship between serum Igs and mucosal Igs, or between VN and ELISA antibodies. All calculations were run with Minitab release 10 *Xtra* software (Minitab, Pa. USA) on a Macintosh computer.

Results

Intranasally administered RSV ISCOMs induce potent IgA response in the local mucosa

In an introductory experiment, β-propiolactone inactivated RSV (s.c.) and live RSV (i.n.) were compared with RSV ISCOMs after i.n. administration for the ability to induce IgA response in the lungs. The inactivated and the live RSV induced low or undetectable levels of local IgA response in the lungs. These formulations were, therefore, not used in the further study.

RSV envelope proteins incorporated into ISCOMs induced high IgA locally in the lungs with absorbances in ELISAs at dilutions down to 1:10,000 which were three times above the background of non-immunized mice (Fig. 1).

Total IgG and IgA concentrations in serum and in extracts from lungs, upper respiratory, genital and intestinal tracts

Total IgG and IgA concentrations were measured in serum and in mucosal organs to facilitate the calculation of RSV-specific antibody responses in arbitrary units based on the correlation to the total concentrations of IgG and IgA, respectively.

The median of total IgG concentrations measured in the quantitative IgG ELISA ranged from 5.680 mg/ml in serum to 0.024 mg/g in the URT. The total IgG concentration in the genital tract was significantly higher than that

Table 1 The total IgG and IgA concentrations (mg/ml or/g, median and range) in serum and in four mucosal organs from RSV ISCOM immunized and nonimmunized mice (*RSV* respiratory syncytical virus, *ISCOM* immunostimulating complex, *URT* upper respiratory tract, *GT* genital tract, *IT* intestinal tract)

	Serum	GT	Lung	IT	URT
IgG	5.680 (0.390–19.610)	0.119 (0.003–0.694)	0.033[a] (0.002–0.676)	0.027[b] (0.004–0.124)	0.024[c] (0.003–0.230)
	Serum	IT	GT	Lung	URT
IgA	0.169 (0.110–0.386)	0.079 (0.002–0.211)	0.030 (0.005–0.135)	0.007 (0.001–0.079)	0.002 (0.000–0.009)

No significant difference was observed between [a–b] ($P = 0.700$), [a–c] ($P = 0.658$) or [b–c] ($P = 0.853$) pairs ($n = 24$). The differences between the other pairs are all significant at least at $P < 0.01$ level

from lungs, intestinal tract and URT, which had similar levels of total IgG concentrations (Table 1).

The highest median of total IgA concentration was detected in serum (0.169 mg/ml) and the lowest (85-fold lower) was in the URT (0.002 mg/g). The differences of total IgA concentrations amongst samples from serum and mucosal organ extracts were all statistically significant (Table 1).

The IgG response to RSV in various mucosal organs

The highest IgG response to RSV was recorded in serum with end-point titers of about 1:90,000, which was 30-fold higher than that measured in mucosal organs (not shown).

The median ratios of anti-RSV IgG versus total IgG were all significantly higher in samples from RSV ISCOM-immunized animals than those from the non-immunized controls. Interestingly, the highest ratios of the RSV-specific IgG/total IgG were recorded in intestinal and genital tracts. The ratios of specific anti-RSV IgG/total IgG in serum were not high, but the lowest background values were also observed for serum. Taken together, mice immunized s.c. and those immunized i.n. with different doses of RSV ISCOMs responded with similar levels of RSV-specific IgG (Fig. 2).

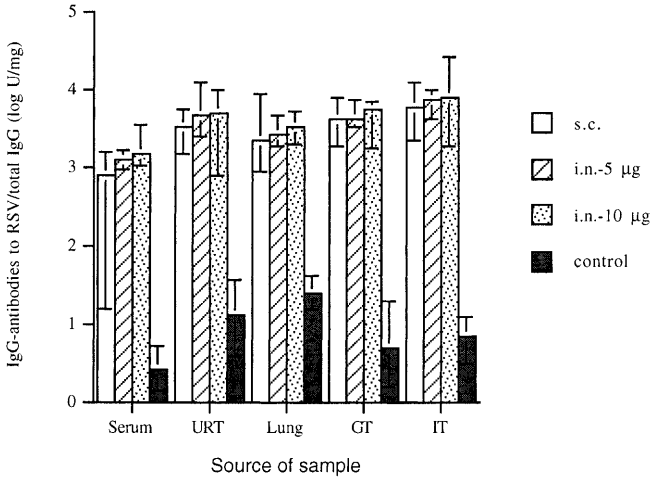

Fig. 2 The IgG response of mice to RSV in serum and in four mucosal organs measured by ELISA and expressed as median ratio with 95% C. I. of IgG anti-RSV to total IgG after two immunizations 6 weeks apart, s.c. with 1 µg (*s.c.*), i.n. with 5 µg (*i.n.-5 µg*) and 10 µg (*i.n-10 µg*) of RSV ISCOMs, and a non-immunized control group. Each group comprised six mice. Blood and organ samples were collected 2 weeks after the second immunization (*URT* upper respiratory tract, *GT* genital tract, *IT* intestinal tract, *C. I.* confidence interval)

IgA response to RSV in various mucosal organs

Doses of 5 and 10 µg ISCOMs induced high IgA responses to RSV of similar levels after two i.n. immunizations in all four mucosal organs tested (Fig. 3). Local responses in the lungs and URT and remote responses in the genital tract the IgA levels were of similar magnitude, while the IgA level to RSV was 3-fold lower in the intestinal tract than in the lungs. The IgA response was about 70-fold lower in serum than in organs as measured by end-point titers (not shown) for both doses, 5 and 10 µg, tested (Figs. 1, 3).

The median ratios of IgA antibody to RSV versus total IgA in sera and in the mucosal organs were all significantly higher than that of the controls and than that induced by s.c. immunization. There was no measurable IgA response after s.c. immunization with RSV ISCOMs (Fig. 3).

Correlations between antibodies to RSV in sera and in the mucosal organs

The origin of antibodies collected from the mucosal organs has to be questioned. To analyze the likelihood of mucosal versus systemic origin, the correlations between the mucosal and serum levels of IgG and IgA were calculated. Strong correlations between the levels of RSV IgG antibody in the sera and in the extracts from the four mucosal organs were found when values for all groups of RSV ISCOM-immunized mice were calculated together, with r ranging from 0.927 (in URT) to 0.892 (in intestinal tract) at a $P < 0.001$ level. In contrast, there was no correlation between levels of anti-RSV IgA in sera and the anti-RSV IgA in the mucosal organ extracts when all i.n. immunized animals were included in the calculation. Both r and P values clearly showed that they were unlikely to be correlated (Table 2).

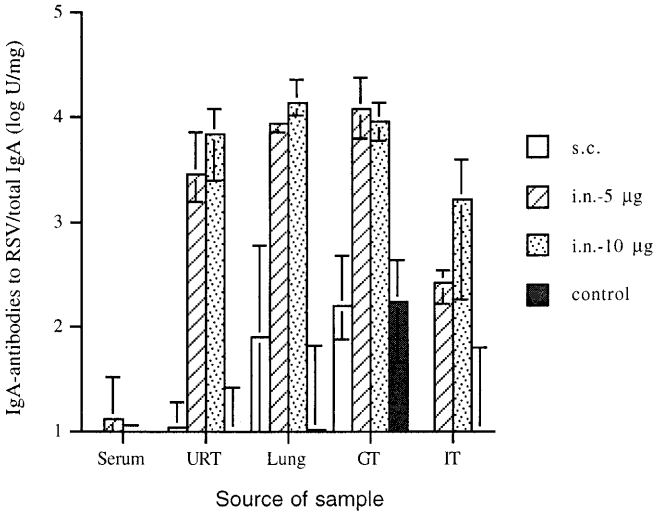

Fig. 3 The IgA response of mice to RSV in serum and in four mucosal organs measured by ELISA, and expressed as median ratio with 95% C. I. of IgA anti-RSV to total IgA after two immunizations 6 weeks apart, s.c. with 1 µg (*s.c.*), i.n. with 5 µg (*i.n.-5 µg*) and 10 µg (*i.n.-10 µg*) of RSV ISCOMs and a non-immunized control group. Each group comprised six mice. Blood and organ samples were collected 2 weeks after the second immunization

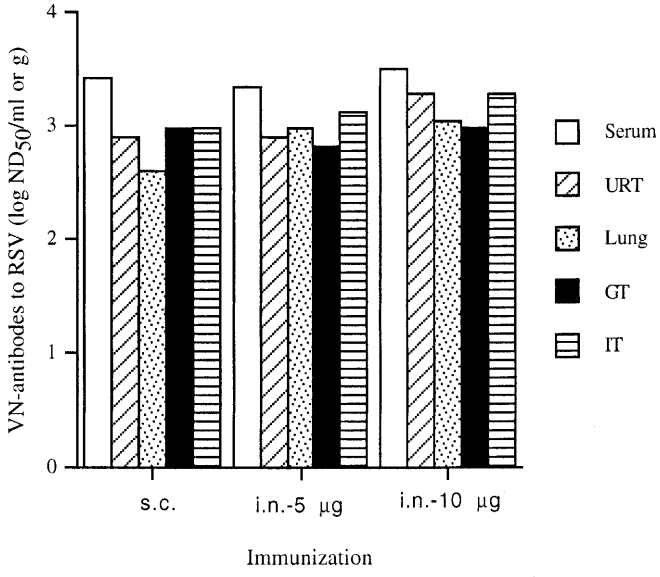

Fig. 4 RSV neutralizing antibody responses in sera, and in mucosal organs 2 weeks after two immunizations of mice 6 weeks apart, s.c. with 1 µg (*s.c.*), i.n. with 5 µg (*i.n.-5 µg*) and 10 µg (*i.n-10 µg*) of RSV ISCOMs. Control mice did not show any virus neutralizing activity at the lowest serum dilution (1:3) and the lowest organ extract dilution (1:6). Pooled samples ($n = 6$) were used for the test

Table 2 Correlations between antibodies to RSV in sera and in the mucosal organs

Antibodies in	Antibodies in sera			
	IgG ($n = 18$)		IgA ($n = 12$)	
	r	*P*	*r*	*P*
URT	0.927	<0.001	0.044	0.898
Lung	0.939	<0.001	–0.297	0.375
GT	0.915	<0.001	0.118	0.729
IT	0.892	<0.001	–0.264	0.461

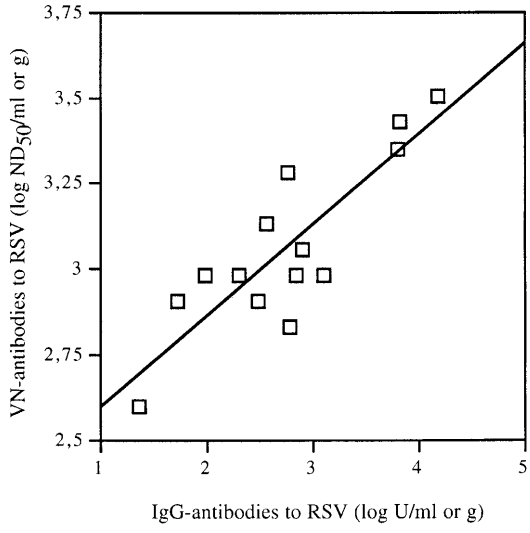

Fig. 5 Correlation between IgG antibodies to RSV (median value in ELISA) and VN antibodies (pooled samples) in sera and in organ extracts (pooled samples) from mice 2 weeks after two immunizations 6 weeks apart with RSV ISCOMs s.c. and i.n. ($n = 12$) (*VN* virus neutralizing)

Both i.n. and s.c. modes of administrations induce VN antibodies to RSV in serum and in mucosal organs, with a high degree of correlation with RSV-specific IgG antibodies

Pooled sera and pooled organ extracts were used in the virus neutralization assay. Generally, the highest VN antibody titers were detected in sera, regardless the route or dose of immunization. Similar levels of VN antibodies were recorded both after i.n. and s.c. administrations in all of the organ extracts, i.e., at the inductive sites (URT and lungs) and the distant effector sites (genital and intestinal tracts). The levels of VN titers were the same after i.n. immunizations with 5 and 10 µg RSV ISCOMs (Fig. 4).

Regardless of the mode of immunization, i.e., s.c. or i.n., a strong correlation was found between VN antibodies (pooled samples) and anti-RSV IgG (median titers of corresponding samples) calculated by Pearson's correlation and regression analysis ($r = 0.854$. $P < 0.001$) (Fig. 5). However, the same calculation run for VN titers versus anti-RSV IgA revealed both a low *r* value (0.032) and a high *P* value (0.996).

RSV envelope proteins in ISCOMs enhance the mucosal IgA response to co-incorporated gp120

The RSV proteins significantly elevated the IgA response to gp120 in the URT and lungs ($P < 0.05$). An enhanced,

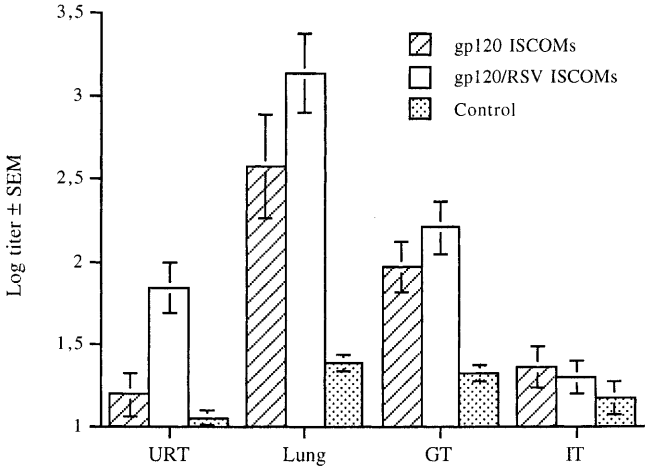

Fig. 6 The IgA response of mice to gp120 of HIV in four mucosal organs measured by ELISA 2 weeks after two immunizations 6 weeks apart with gp120 ISCOMs (10 μg/mouse), gp120/RSV ISCOMs (10 μg/mouse) (n = 10)

but not significant, IgA response to gp120 was also recorded in the genital tract. In the intestinal tract, no enhancement of IgA response to gp120 was recorded (Fig. 6).

Discussion

The difficulty in eliciting mucosal immunity with non-replicating antigens is well established. However, a mucosal immunity would be most desirable because of the invading routes of pathogens. Developments in the area of mucosal adjuvants, encompassing CT(B) [5], LT(B) [9] or mutants of CT [37] and LT [15], and to a less extent, stable microsphere systems [3], digestible micro-carriers and bioadhesive polymers [4], have improved the local and systemic immune responses to mucosally administered antigens. However, all these have shortcomings.

ISCOMs have been extensively tested as immunological carriers for numerous pathogens, proteins and peptides, but mostly explored for systemic immunizations [21]. Recent studies on mucosal applications have shown promise for ISCOMs as carriers for mucosal immunization [22, 23, 24, 30]. In accord with a previous study [11], this study showed that an i.n. administration of a comparatively low dose, i.e., 5 μg/mouse RSV ISCOMs administered twice, induced a surprisingly high IgA response to RSV not only locally, i.e., in the URT and lungs, but also in the remote mucosal organs, i.e., the intestinal and the genital tracts. The highest RSV IgA antibody to total IgA ratios were detected in the lungs and in the genital tract with higher background values for the latter, indicating that the local inductive sites (lungs and URT) may have higher true IgA levels than the two remote effector sites, as expected [26]. The i.n.-introduced ISCOMs also induced a strong systemic antibody response, mainly IgG, which was comparable to that induced by s.c. administration. A higher i.n. dose of 10 μg/mouse did not further increase the mucosal and serum antibody responses, indicating that the ceiling was reached by a 5-μg dose of ISCOMs and showing the potency of the ISCOMs as a mucosal delivery system. The IgG responses were also of similar magnitude in mucosal organs after i.n. and s.c. administration.

Table 3 Comparison of total Igs (IgG+IgA) between RSV ISCOM immunized and nonimmunized groups [*s.c.* subcutaneous immunization with RSV ISCOMs (1 μg/mouse), *i.n.-5 μg* intranasal immunization with RSV ISCOMs (5 μg/mouse), *i.n.-10 μg* i.n. immunization with RSV ISCOMs (10 μg/mouse)]

Compared with control	Immunization		
Ig in	s.c.	i.n.-5 μg	i.n.-10 μg
Serum	*	*	*
URT	NS	*	*
Lung	NS	*	*
GT	*	*	*
IT	NS	NS	NS

* Significant at least at $P<0.05$ level, *NS*: not significant

Apart from the intestinal tract, the mucosal surfaces of mice immunized i.n. with ISCOMs had significantly higher total immunogloblin contents (Table 3), indicating a potent mucosal stimulation of the lymphatic tissue by ISCOMs, which is in accord with that found by parenteral immunization [31]. The increase of the total immunoglobulins was mainly due to increased IgA levels both at the inductive sites (URT and lung) and at the effector site (genital tract), but not in the digestive tract, possibly reflecting a high immunoglobulin secretion by natural stimulation in the digestive tract. s.c. immunization did not induce increased mucosal immunogloblin levels, or elevate the RSV-specific mucosal IgA response. The significant immunoglobulin increase in the genital tract after s.c. immunization might indicate the efficiency of IgG antibody transudation from blood to this organ, as stated by Rosenthal et al. [28].

The IgG responses, expressed as RSV-specific IgG versus total IgG, were of similar magnitude in the four mucosal organs, with comparatively lower background values than those for IgA in the genital and intestinal tracts.

The strong correlations between serum IgG and IgG in mucosal organs are in agreement with other reports [25]. The IgG in organs is likely to be transudated from blood. The strong correlation between IgG and VN antibodies suggested that the VN capacity in the mucosal organs was from IgG rather than from IgA, which is consistent with the observations by Kimman et al. [14] that the presence of bovine RSV-specific IgA was not correlated with neutralizing activity in vitro. It should, however, be noted that non-neutralizing IgA antibodies may have protective value; it has been reported that some non-neutralizing IgA mAb can resolve virus infection [28]. Therefore, the biological functions of the significant amount of IgA induced by i.n. administration with RSV ISCOMs need further investigation.

Comparisons between the various modes of mucosal immunizations with different antigens [7, 8, 29, 36] show that i.n. administration is possibly one of the most effective modes for induction of a common mucosal immune response. On the other hand, the genital tract is a poor inductive site but a good effector site, as shown here and by others [28]. The increase of anti-RSV antibodies encompassing RSV neutralizing antibodies detected in the genital tract of mice after i.n. immunization indicates that ISCOMs efficiently target antibody responses to this organ. This ability of RSV ISCOMs to elevate mucosal IgA responses to gp120 of HIV in local sites and in the remote genital tract when administered i.n. suggests that the potential for such a gp120/RSV ISCOM formulation might be an interesting concept in vaccine development forAIDS and other sexually transmitted diseases.

Currently studies are also in progress to characterize localized B and T cell responses in various mucosal sites after i.n. immunization with RSV ISCOMs or after challenge with live RSV since these data are crucial for RSV immunization.

Acknowledgements This study was supported by grants from the Swedish Research Council for Engineering Sciences. Special thanks are due to Dr Aud K. H. Berstad for valuable discussion.

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