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# Tissue distribution of mucosal antibody-producing cells specific for respiratory syncytial virus in severe combined immune deficiency (SCID) mice engrafted with human tonsils

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## SUMMARY

Groups of C.B-17 SCID mice were reconstituted intraperitoneally with human tonsillar mononuclear cells (hu-TMC) from children seropositive for antibody to respiratory syncytial virus (RSV) and subsequently challenged intraperitoneally with inactivated RSV or sham-immunized. The synthesis and the distribution characteristics of human antibody to RSV in various murine tissues were studied using an enzyme-linked immunospot assay (ELISPOT). No specific antibody was observed in sham-immunized animals. In contrast, mice engrafted with hu-TMC exhibited the appearance of specific human antibody secreting cells (hu-ASC) after i.p. immunization with inactivated RSV. RSV-specific hu-ASC were detected only in animals engrafted with cells from donors seropositive for antibodies to Epstein–Barr virus. Hu-TMC engrafted mice showed RSV-specific IgM and, in lower numbers, IgG hu-ASC in several tissues including the lungs. Numbers of RSV-specific IgA hu-ASC were low, however, and detected only in the lung. No RSV-specific hu-ASC were detected in the intestine. These data demonstrate for the first time that hu-TMC–SCID chimeras respond to immunization with viral antigen. Furthermore, the results suggest that hu-TMC engraft in lungs but not in the intestinal tissue.

**Keywords** scid mice chimera tonsillar cells respiratory syncytial virus antibody production

## INTRODUCTION

Mice with SCID exhibit an autosomal genetic defect that prevents the rearrangement of antigen receptor genes in lymphoid precursors resulting in the lack of functional lymphocytes (Dorshkind *et al.*, 1984). Recent investigations have demonstrated that human fetal lymphoid tissue and peripheral blood lymphocytes (hu-PBL) from adults can survive in SCID mice (McCune *et al.*, 1988; Mosier *et al.*, 1988). The SCID mouse–human chimeras have provided an *in vivo* model for evaluation of immunopathogenesis and treatment of human diseases such as infection with HIV, autoimmune processes, and malignancies (Namikawa *et al.*, 1988; Kamel-Reid *et al.*, 1989; Krams, Dorshkind & Gershwin, 1989; Cannon *et al.*, 1990; Duchosal *et al.*, 1990; McCune *et al.*, 1990). However, the ability of human cells to produce specific antiviral antibodies upon antigen challenge within the SCID mouse has not been reported so far.

The present studies were undertaken to determine whether human mucosal cells, i.e. tonsillar mononuclear cells (hu-TMC), transferred to SCID mice exhibit differences in their ability to engraft in various mucosal tissues, namely lung *versus* intestine, and to mount an antibody response specific for a virus commonly associated with respiratory mucosa. We tested various mucosal tissues for the appearance of human antibody secreting cells (hu-ASC), employing respiratory syncytial virus (RSV), a ubiquitous pathogen with an exquisite tropism for the respiratory epithelium (McIntosh & Chanock, 1985), which induces severe pneumonia and bronchiolitis in infants in association with significant alterations in mucosal immunologic reactivity (McIntosh *et al.*, 1973; Welliver, Kaul & Ogra, 1979, 1980; Welliver *et al.*, 1981). We have shown that hu-TMC injected intraperitoneally respond to i.p. challenge with viral antigen with antibody production. RSV-specific hu-ASC appear in various tissues including the lung, but not in the intestine. Interestingly, the IgA production was observed only in lung.

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## MATERIALS AND METHODS

### *Human lymphocytes*

Human tonsils were obtained from 18 subjects during therapeutic tonsillectomy. The mean age of the donors (12 males, six

**Table 1.** Experimental protocol and immunization groups employed in this study

| Group | | Day 0 | | Day 28–35 |
|---|---|---|---|---|
| | | i.p. reconstitution with human cells | | i.p. immunization with |
| No. | $n$ | Type | Number | |
| 1 | 30 | TMC | $50 \times 10^6$ | RSV* |
| 2 | 20 | PBL | $20 \times 10^6$ | RSV* |
| 3 | 4 | TMC | $50 \times 10^6$ | — |
| 4 | 4 | — | — | — |
| 5 | 4 | Irradiated TMC or PBL | $50 \times 10^6$ | — |

TMC, tonsillar mononuclear cells; PBL, peripheral blood lymphocytes; *$10^6$ plaque-forming units of u.v.-inactivated virus; —, received no cells or virus for reconstitution or immunization. All mice were killed during days 42–49.

females) was 7·7 years (range 3–20). All donors were known to be seropositive for IgG antibody against RSV. As a control, hu-PBL were obtained from the same donors. Separation of hu-TMC and hu-PBL was done by Ficoll–Hypaque centrifugation (Histopaque 1077, Sigma, St Louis, MO) (Okamoto *et al.*, 1988).

*Mice*

C.B-17 SCID mice (Bosma, Custer & Bosma, 1983) were originally obtained from the Fox Chase Cancer Center, Philadelphia, PA, and thereafter bred in isolation in our own animal unit in laminar flow facility. The animals were maintained in microisolator cages (Labproducts Inc., Maywood, NJ). Food, water and bedding were sterilized. Only non-leaky animals (shown not to produce immunoglobulin) were included in the experiments.

*Experimental design*

Groups of male SCID mice 4–8 weeks old were reconstituted and immunized according to the protocol listed in Table 1. Group 1 consisted of 30 SCID mice reconstituted intraperitoneally with $50 \times 10^6$ hu-TMC. Groups 2 to 5 represented controls and included sham- and RSV-immunized animals, and mice reconstituted with hu-PBL. Group 2 consisted of 20 animals injected with $20 \times 10^6$ hu-PBL. The use of lower numbers of hu-PBL was necessitated by the limited volume of blood available from the young children undergoing tonsillectomy. After 4–5 weeks, the mice in groups 1 and 2 were immunized intraperitoneally with $10^6$ plaque-forming units of RSV inactivated under u.v.-light (30 min at a distance of 8 cm) immediately before injection. Group 3 consisted of four SCID mice reconstituted with $50 \times 10^6$ hu-TMC intraperitoneally and sham-immunized 4 weeks later with saline intraperitoneally. Group 4 included four additional mice sham-reconstituted and sham-immunized intraperitoneally with saline in a similar manner as in group 3. Engraftment of human cells was demonstrated by the presence of human immunoglobulin secreting cells (hu-ISC) in the animals when killed. Mice of group 5 were injected with lethally irradiated (2000 rad) $50 \times 10^6$ hu-TMC (two mice) or $50 \times 10^6$ hu-PBL (two mice).

*Specimen collection*

All SCID mice were killed 2 weeks after immunization. Following final bleeding by heart puncture, peritoneal cells were collected by lavage with 5 ml of Hanks' balanced salt solution (HBSS) (GIBCO, Grand Island, NY). Subsequently, thorax and abdomen were opened and lungs (including lower trachea and mediastinal lymph nodes), liver, spleen, and small intestine were placed into separate Petri dishes containing HBSS, cut into small pieces, teased, and passed through wire mash. Cells from bone marrow were collected by flushing repeatedly the cavities of both femora in each animal. Mononuclear cell preparations enriched for human cells were obtained by centrifugation over Histopaque 1077. After two washings in RPMI 1640 (GIBCO) supplemented with 5% fetal calf serum (FCS) (GIBCO), the cell suspension was adjusted to $10^6$ viable cells/ml. Viability of the cells was determined by the trypan blue exclusion method.

*Antibody to Epstein–Barr virus*

The serum IgG antibody status of the human donors to Epstein–Barr virus (EBV) viral capsid antigen was assessed using an indirect immunofluorescence assay (Zeus Scientific, Raritan, NJ) (Henle, Henle & Horowitz, 1974).

*RSV antigen preparation*

The long strain of RSV (ATCC VR-26) was grown in HEp-2 cells as previously described (Wong *et al.*, 1985). Briefly, cell monolayers were infected with the virus. When the cytopathic effect reached 80%, the monolayers were disrupted, sonicated and clarified at 10 000 *g* for 20 min at 4°C. The virus stock was aliquoted and stored at −70°C until further use. Titration of the virus was done by plaque assay on HEp-2 cell monolayers (Schmidt, 1982).

RSV antigen for the detection of specific hu-ASC was prepared by partial purification of virus by means of ultracentrifugation on sucrose gradients (Ueba, 1980). Uninfected HEp-2 cells were processed similarly and employed as controls.

*Detection of RSV-specific human antibody secreting cells (hu-ASC)*

Human ASC specific for RSV were detected by adapting a solid phase ELISPOT assay previously described by Czerkinsky *et al.* (1988). In brief, single microtitre wells of nitrocellulose bottomed plates (Millipore, Bedford, MA) were filled with 100 μl of RSV and control wells with uninfected HEp-2 cells diluted in PBS to a protein concentration of 5 μg/ml. The optimal coating dilution was determined in preliminary experiments. Coated plates were kept overnight at 4°C. Unsaturated binding sites on the nitrocellulose were blocked by incubation of the plates with 200 μl of RPMI 1640 with 5% FCS per well at 37°C for at least 30 min in a humidified atmosphere with 5% $CO_2$. Each well was filled with $10^5$ mononuclear cells in 100 μl RPMI FCS 5%. The samples were run in duplicate. After a 2-h incubation at 37°C in a humid atmosphere with 5% $CO_2$, the plates were washed three times with PBS and three times with PBS containing 0·05% Tween 20 (Sigma) and immersed in the latter buffer for 5 min. Horseradish-peroxidase conjugated heavy chain specific rabbit anti-human IgG, IgA, or IgM (Dakopatts, Copenhagen, Denmark) were used to visualize human immunoglobulin adhering

to the wells. The substrate for colour development consisted of 3-amino-9-ethyl-carbazole (Sigma), dimethylformamide (Aldrich, Milwaukee, WS), acetate buffer, and $H_2O_2$ (Czerkinsky *et al.*, 1988). The reaction was stopped after 10 min with tap water. Spots were counted under low magnification (40–60 ×) using a stereomicroscope equipped with a circumferencial epi-illumination. The mean of the duplicate values was calculated. The counts obtained in wells coated with uninfected HEp-2 cells, if any, were deducted from the values in wells coated with partially purified RSV. The formation of spots was inhibitable by cycloheximide treatment of the cells as described previously (Czerkinsky *et al.*, 1988), thus confirming that the spots represent antibody actively secreted and not antibody bound to cells and passively deliberated.

The specificity of the antisera used was verified in control experiments using (a) hu-TMC, hu-PBL, mononuclear cells from various tissues of non-reconstituted SCID mice and (b) uncoated but blocked wells.

*Detection of total human immunoglobulin secreting cells (hu-ISC)*

To assess engraftment, polyclonal human IgG, IgA, and IgM secreting cells were quantified by an ELISPOT assay (Czerkinsky *et al.*, 1988) modified as a capture test. Affinity purified rabbit anti-human $\alpha$, $\gamma$, and $\mu$ chain specific antisera (Dakopatts) were used for capture.

*Statistics*

Numbers of hu-ASC and hu-ISC were expressed as mean ± s.e.m. for each organ and each isotype. The means ± s.e.m. of ratios of RSV-specific hu-ASC over hu-ISC were calculated from the ratios obtained for individual animals and expressed in per cent. Proportional statistics for non-parametric testing were performed using Fisher's exact test (Kramer, 1988). Values of $P < 0{\cdot}01$ were regarded as significant.

## RESULTS

*Distribution of RSV-specific human antibody secreting cells (hu-ASC)*

Intraperitoneal transfer of hu-TMC and immunization with inactivated RSV in group 1 animals resulted in varying frequency in the appearance of RSV-specific hu-ASC in different systemic *versus* mucosal sites. Only SCID mice injected with cells from EBV antibody-positive donors exhibited RSV-specific hu-ASC (13 out of 17 *versus* 0 out of 13; $P < 0{\cdot}005$). Therefore, all subsequent experiments reported here are based on group 1 animals receiving cells from EBV antibody-positive donors.

In group 1, 11 SCID mice receiving hu-TMC from six donors responded to RSV immunization with antibody production, two out of four SCID mice injected with hu-TMC from two donors, and none out of two SCID mice receiving hu-TMC of one donor (Table 2). Among the EBV antibody-positive donors there was no significant difference in the titres of antibodies to RSV.

Transfer of hu-TMC and subsequent challenge with RSV resulted in the development of hu-ASC of IgM and, less frequently, IgG class in the peritoneum, liver, bone marrow and spleen as shown in Fig. 1. However, RSV IgA hu-ASC were not detected at these sites. No hu-ASC producing IgG specific for RSV were detected among the peritoneal cells (Fig. 1), i.e. at the site of injection and immunization. RSV-specific IgA hu-ASC activity was detected only in the lungs (Fig. 1). As shown in Fig. 1, the lungs of animals reconstituted with hu-TMC showed furthermore IgM hu-ASC and were more often positive for RSV hu-ASC of the IgG isotype than corresponding liver, spleen and bone marrow. Interestingly, no RSV-specific hu-ASC were detected in the intestines.

**Table 2.** Donor related success of RSV immunization in hu-TMC SCID mice

| EBV antibody-positive hu-TMC donor | hu-TMC SCID mice positive for RSV hu-ASC following | |
|---|---|---|
| | RSV immunization | Sham immunization |
| 1 | 2/2 | 0/1 |
| 2 | 2/2 | 0/1 |
| 3 | 2/2 | 0/1 |
| 4 | 2/2 | 0/1 |
| 5 | 2/2 | ND |
| 6 | 1/1 | ND |
| 7 | 1/2 | ND |
| 8 | 1/2 | ND |
| 9 | 0/2 | ND |
| Total 9 | 13/17 | 0/4 |

SCID mice were injected $50 \times 10^6$ hu-TMC intraperitoneally; after 4–5 weeks the mice were immunized with $10^6$ plaque-forming units RSV inactivated by u.v. or sham-immunized, and killed 2 weeks later. RSV-specific hu-ASC were detected by ELISPOT. ND, not done.

The mean numbers of RSV-specific hu-ASC detected in various organs of reconstituted SCID mice are presented in Table 3. Numbers of IgM and IgG hu-ASC in lungs and the liver were more than three-fold higher than in the spleen and bone marrow. RSV-specific IgM hu-ASC were most frequent in the peritoneum, the site of initial reconstitution and subsequent RSV immunization.

Reconstitution with hu-PBL from EBV antibody-positive donors (group 2; controls) resulted in the appearance of IgM and IgG RSV-specific hu-ASC in all systemic tissues tested. No RSV-specific IgA hu-ASC activity was observed in the lung or intestine. Minimal IgA hu-ASC reactivity was only observed in the peritoneum and liver after transfer of hu-PBL.

No RSV-specific hu-ASC were detected in sham-immunized and/or sham-reconstituted animals (groups 3–5). The sham-immunized SCID mice of group 3 received hu-TMC from four EBV antibody-positive donors; cells from the same donors responded with specific antibody production in eight RSV challenged chimeras (Table 2).

*Hu-ISC as a measure of engraftment of hu-TMC in SCID mice*

The ability of hu-TMC SCID mouse chimera tissues to respond to RSV challenge may reflect the extent of engraftment of cells of the human B cell line. Therefore, engraftment of human cells was evaluated by the use of ELISPOT for hu-ISC. The transfer of hu-TMC resulted in engraftment in all 17 SCID mice in group 1 injected with cells from EBV antibody-positive donors and in six of 13 mice receiving cells from EBV antibody-negative individuals ($P < 0{\cdot}01$). Hu-ISC numbers were lower in mice

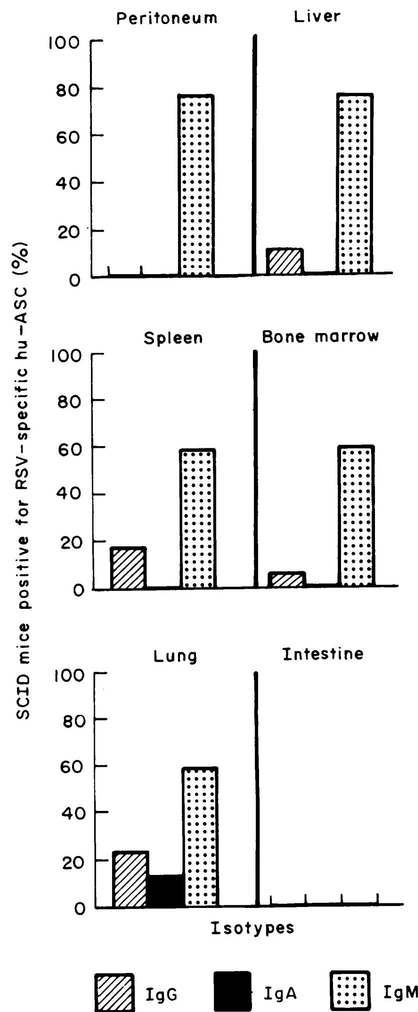


**Fig. 1.** Frequency of RSV-specific hu-ASC in hu-TMC SCID mice. The mice ($n=17$) were engrafted with $50\times10^6$ hu-TMC each from EBV antibody-positive donors, immunized intraperitoneally with $10^6$ plaque-forming units of u.v.-inactivated RSV 4–5 weeks after reconstitution, and killed 2 weeks later. RSV-specific hu-ASC were detected by ELISPOT.

injected with cells from EBV antibody-negative donors than in mice receiving cells from EBV antibody-positive donors.

As shown in Table 4, chimeras responding to RSV with antibody production showed in general higher numbers of IgM hu-ISC than chimeras unresponsive to RSV. Unfortunately, due to the large s.e.m., these differences were statistically not significant.

In group 2, transfer of hu-PBL led to engraftment in peritoneum, liver, spleen, and bone marrow in 10 of 12 animals receiving cells from EBV antibody-positive donors and in four of eight mice inoculated with cells from EBV antibody-negative individuals. Only few hu-ISC were seen in lungs and no hu-ISC were observed in the intestines. The four mice of group 3 reconstituted with hu-TMC from EBV antibody-positive donors demonstrated engraftment to a similar extent as observed in group 1 animals. No hu-ISC were detected in the sham-reconstituted mice in group 4. SCID mice injected with lethally irradiated human lymphocytes (group 5) did not show engraftment.

**Table 3.** RSV-specific hu-ASC recovered from 13 hu-TMC SCID mice

| Tissue | IgG | IgA | IgM |
|---|---|---|---|
| Peritoneum | 0 | 0 | 1027·8 ± 666·4 |
| Non-mucosal | | | |
| Liver | 22·3 ± 19·1 | 0 | 478·2 ± 334·4 |
| Spleen | 1·5 ± 1·0 | 0 | 123·6 ± 73·1 |
| Bone marrow | 0·8 ± 0·8 | 0 | 30·9 ± 10·7 |
| Mucosal | | | |
| Lung | 6·9 ± 3·8 | 2·7 ± 2·7 | 443·6 ± 285·2 |
| Intestine | 0 | 0 | 0 |

SCID mice were injected intraperitoneally with $50\times10^6$ hu-TMC; after 4–5 weeks the mice were immunized intraperitoneally with $10^6$ plaque-forming units of RSV inactivated by u.v. and killed 2 weeks later. Enumeration of RSV-specific hu-ASC was done by ELISPOT. Each value indicates mean ± s.e.m. of RSV-specific hu-ASC per $10^6$ mononuclear cells enriched for human mononuclear cells by centrifugation of single cell suspension over Ficoll–Hypaque (density 1077 g/ml).

*Ratios of RSV-specific human antibody to total immunoglobulin isotypes*

The proportions of RSV-specific hu-ASC in relation to the corresponding number of total isotype-specific hu-ISC detected in various tissues were calculated for each animal. The means of these proportions are presented in Table 5. In animals engrafted with hu-TMC the ratios of specific hu-ASC for IgG, IgA, and IgM were higher in lung, spleen, and bone marrow than in peritoneum (Table 5). In these animals, RSV-specific hu-ASC of the IgM isotype constituted the highest proportion of total hu-ISC. Significantly, however, the highest ratio of RSV-specific IgM hu-ASC was observed in the lungs (Table 5). The lungs also exhibited a higher ratio of RSV-specific hu-ASC of the IgG isotype than other organs. IgA hu-ASC reactivity was, even though modest when expressed as percentage of IgA hu-ISC (0·42%), restricted to the lung tissue.

## DISCUSSION

The study reported here demonstrates the ability of human mucosa-associated lymphocytes after transfer into SCID mice to respond with specific antibody production to challenge with a mucosa-associated pathogen. RSV-specific hu-ASC were detected in the peritoneal cavity, the primary site of reconstitution and immunization, and in other distant organs after exposure to RSV. Hu-TMC transferred to SCID mice were not found to produce spontaneously specific antibody to RSV, even though the donors had circulation antibodies to RSV (Table 2). After engraftment with hu-TMC and antigenic challenge, specific hu-ASC were detected with high frequency in lungs but were conspicuously absent in the intestine. These results are in good agreement with the notion of distinct bronchus associated (BALT) and gut associated lymphoid systems (GALT) (Sminia, van der Brugge-Gamelkoorn & Jeurissen, 1989).

In earlier studies, SCID mice reconstituted with human lymphocytes were inoculated with live HIV or EBV, but the

**Table 4.** hu-ISC recovered from hu-TMC SCID mice immunized with RSV

| | hu-TMC SCID mice | | | | | |
|---|---|---|---|---|---|---|
| | Positive for RSV-specific hu-ASC ($n=13$) | | | Negative for RSV-specific hu-ASC ($n=4$) | | |
| Tissue | IgG | IgA | IgM | IgG | IgA | IgM |
| Peritoneum | 2370±1130 | 681±615 | 16961±6799 | 5237±492 | 275±216 | 568±396 |
| Non-mucosal | | | | | | |
| Liver | 1211±449 | 722±358 | 10083±4426 | 6168±4638 | 643±466 | 2073±1977 |
| Spleen | 268±117 | 154±84 | 2419±807 | 1008±683 | 123±56 | 68±29 |
| Bone marrow | 89±37 | 25±11 | 558±212 | 183±80 | 13±7 | 10±7 |
| Mucosal | | | | | | |
| Lung | 1255±618 | 361±146 | 5467±2646 | 3028±1137 | 360±232 | 488±299 |
| Intestine | 0 | 0 | 0 | 0 | 0 | 0 |

SCID mice were injected, immunized and killed as described in the legend to Table 3. Enumeration of hu-ISC was done by ELISPOT. Each value indicates mean±s.e.m. of hu-ISC per $10^6$ mononuclear cells enriched for human cells by centrifugation of single cell suspension through Ficoll–Hypaque (density 1077 g/ml).

**Table 5.** Distribution of RSV-specific human ASC compared to total isotype-specific hu-ISC recovered from 13 hu-TMC SCID mice

| Tissue | IgG | IgA | IgM |
|---|---|---|---|
| Peritoneum | 0 | 0 | 3·59±1·53 |
| Non-mucosal | | | |
| Liver | 0·24±0·14 | 0 | 2·69±1·05 |
| Spleen | 0·65±0·47 | 0 | 4·01±2·19 |
| Bone marrow | 0·11±0·11 | 0 | 5·13±1·84 |
| Mucosal | | | |
| Lung | 1·64±1·53 | 0·42±0·42 | 5·73±2·33 |
| Intestine | —* | — | — |

SCID mice were injected, immunized and killed as described in the legend to Table 3. Enumeration of hu-ASC and of hu-ISC was done by ELISPOT. Each value indicates mean±s.e.m. of the ratio RSV-specific hu-ASC/hu-ISC calculated for each animal (in per cent).

* No hu-ASC or hu-ISC detected.

antibody response to the pathogens was not reported (Namikawa *et al.*, 1988; Cannon *et al.*, 1990; McCune *et al.*, 1990). The ELISPOT used here is efficient in detecting specific antibody responses. The immune response to viral pathogens restricted to the respiratory tract was seen among the mucosal tissues studied in the lungs but not in the intestines of animals engrafted with hu-TMC. The mechanism underlying the selectivity in mucosal engraftment between lungs and the intestines for hu-TMC remains to be determined. In an independent study, hu-TMC donor cells were traced in the hu-TMC SCID mouse chimera using a supravital nuclear stain. Again human cells were detected in lungs but not in intestine (Nadal *et al.*, 1991).

In RSV-challenged hu-TMC SCID mice, IgG and IgM hu-ASC were detected in significantly larger numbers than IgA in the tissues studied. It is known from a comparative *in vitro* study on influenza virus, another respiratory tract pathogen, that after *in vitro* stimulation, hu-TMC produce high levels of specific IgG, IgA, and IgM (Yarchoan *et al.*, 1985). In the same study, precursor frequency analysis for IgA B cells suggested a high frequency of memory cells in tonsillar tissue. On the other hand, it is possible that human lymphoid cells harbour more precursor cells for the IgM than for other isotypes. A preferentially better survival of human IgM memory or effector cells in SCID mice than of other isotype-specific memory cells may also have contributed to our finding. Germinal centres clearly contribute to secondary plasma cell responses through their role in the generation of B cell memory (MacLennan *et al.*, 1990). In human tonsils nearly 60% of the cells isolated from germinal centres express immunoglobulin which is almost exclusively IgG (Liu *et al.*, 1989). Analysis of hu-TMC has also demonstrated a high death rate of germinal centre derived B cells when not stimulated (Liu *et al.*, 1989). In rats the life-span of transferred memory B cells in the absence of antigen has been reported to be 3–4 weeks (Gray & Leanderson, 1990). The mice in the present study were challenged with RSV 4–5 weeks after transfer of human cells. Thus, the results observed here may reflect the faster death rate of B cells derived from germinal centres. Finally, presence of inhibitor substance(s) in SCID mice (Fernandez-Botran & Vita, 1990) and/or lack of cytokine(s) (Beagley *et al.*, 1989; Kishimoto & Hirano, 1989) or other T cell help (Kawanishi, Saltzman & Strober, 1982, 1983a,b; Kiyono *et al.*, 1982, 1984, 1985; Mayer, Fu & Kunkel, 1982; Mayer, Posnett & Kunkel, 1985) could have inhibited the proliferation of other specific hu-ASC. In preliminary studies in animals reconstituted with hu-TMC, $CD3^+$ cells were seen in the peritoneal cavity, spleen, and lungs with a frequency of 6–40% of total mononuclear cells, whereas hu-PBL reconstituted mice showed a higher proportion of $CD3^+$ cells in the peritoneal cavity, but no $CD3^+$ cells in the lungs (Chen *et al.*, 1991).

In the present studies, specific hu-ASC for RSV were found only after transfer of cells from EBV antibody-positive donors. In a recent report, extensive homology of the murine cytokine synthesis inhibiting factor, provisionally termed 'interleukin 10', with the EBV gene BCRFI was demonstrated (Moore *et al.*,

1990). Cytokine synthesis inhibitory factor seems to enhance antibody synthesis through inhibition of interferon and of other cytokines (Moore *et al.*, 1990). Thus, it seems that the EBV status of the donor may be an important factor influencing the engraftment of immunologically reactive cells in SCID mice, both hu-TMC and hu-PBL.

Even though all nine EBV antibody-positive hu-TMC donors were positive for RSV antibodies at comparable titres, the frequency of antibody production in chimeras in response to RSV immunization differed (Table 2). All mice reconstituted with hu-TMC from six donors, 50% of mice reconstituted with hu-TMC from two donors, and none of the two mice reconstituted with hu-TMC from one donor responded to RSV immunization. The mechanism underlying these differences is difficult to understand, since all mice tested showed engraftment of hu-TMC.

It is of particular interest that hu-TMC derived RSV-specific hu-ASC appeared in high numbers in the lung (Table 3), but not in the intestine. The pattern of hu-ASC tissue distribution within mucosal tissues observed in the present study seemed to reflect the tissue tropism of the pathogen to which the hu-ASC were reactive. However, presence of RSV in the respiratory tract itself was not necessary, since RSV was administered intraperitoneally. It is known from an earlier study that fetal lymphocytes which are presumably deficient in memory cells do not exhibit organ selective recirculation (Cahill *et al.*, 1979). In contrast, antibody-producing blast cells, or their immediate precursors, exhibit a different behaviour. After immune challenge more differentiated cells enter the circulation, home to distant sites, and remain associated with the target tissue (Husband & Gowans, 1978; Pierce & Cray, 1982). Various studies have demonstrated the independence of homing to the target tissue relative to the site of antigenic exposure (Parrott & Fergusson, 1974; Hall, Hopkins & Orlans, 1977; Husband & Gowans, 1978). Such homing of antigen-sensitized cells showed stronger tissue specificity when the cells were exposed to a secondary antigenic challenge (Pierce & Cray, 1982). Since all cell donors in the present study were known to be sensitized to RSV, our findings suggest that engraftment patterns are determined to some extent by the tissue characteristics of the site of primary antigen interactions. Studies using human intestine-derived lymphoid cells to engraft SCID mice are currently in progress.

These observations provide evidence that the hu-TMC SCID mouse is a useful model for the study of human immune responses to microbes infecting the mucosal tissues of the respiratory tract.

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