The Journal *of* Immunology

Intranasally Induced Immunological Tolerance Is Determined by Characteristics of the Draining Lymph Nodes: Studies with OVA and Human Cartilage gp-39

Danielle A. W. Wolvers, Christina J. J. Coenen-de Roo, Reina E. Mebius, Maarten J. F. van der Cammen, Felicia Tirion, André M. M. Miltenburg and Georg Kraal

---

References   This article **cites 20 articles**, 8 of which you can access for free at:
http://www.jimmunol.org/content/162/4/1994.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription   Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions   Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts   Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Intranasally Induced Immunological Tolerance Is Determined by Characteristics of the Draining Lymph Nodes: Studies with OVA and Human Cartilage gp-39

Danielle A. W. Wolvers,[1]* **Christina J. J. Coenen-de Roo,[†]** **Reina E. Mebius,***
Maarten J. F. van der Cammen,* Felicia Tirion,* André M. M. Miltenburg[†], and Georg Kraal[2]*

Mucosal tolerance is a naturally occurring immunological phenomenon that prevents harmful inflammatory responses to ingested or inhaled environmental, predominantly nondangerous, Ags. The nasal mucosa is an extremely efficient compartment in the induction of immunological tolerance which can be exploited in Ag-specific treatment of autoimmune disease. With the use of a model Ag (OVA) and an Ag implicated in the autoimmune disease rheumatoid arthritis (human cartilage gp-39), we here show in a mouse model that the superficial cervical and internal jugular lymph nodes that drain the nasal mucosa are instrumental in the induction of tolerance. Removal of these lymph nodes abrogates tolerance induction, which can be restored by transplantation of superficial cervical lymph nodes, but not of peripheral lymph nodes. The results indicate that lymph nodes that directly drain the nasal mucosa constitute a unique microenvironment which favors the induction of immunological tolerance. *The Journal of Immunology,* **1999, 162: 1994–1998.**

ucosal tolerance is a naturally occurring immunolog-ical phenomenon that prevents harmful inflammatory responses to nondangerous proteins such as food com-ponents. Understanding the mechanisms that lead to mucosal tol-erance might provide ways to treat allergies and autoimmune dis-eases such as rheumatoid arthritis (RA)[3]. Mucosal sites that are suitable for tolerance induction are both the gastro-intestinal tract (1, 2) and the airway system (3–6). Tolerance can involve clonal deletion, clonal anergy, and active T cell-mediated suppression (7). When tolerance is induced via the intranasal route, active suppres-sion is involved as demonstrated by adoptive transfer of spleno-cytes (8). It was demonstrated that nasal tolerance leads to efficient down-regulation of both DTH, provoking Th1 cells, and IgE, sup-porting Th2 cells (9, 10). However, the sites and conditions for the generation of active suppressor populations via the nose are unclear.

It has been shown that Ag delivered via the nasal mucosa leads to activation in the cervical lymph nodes in the neck region and spleen, as can be inferred from the production of cytokines such as IL-3, granulocyte-macrophage CSF, IL-2, and IFN-γ in these or-gans shortly upon Ag delivery in the nose (11). Moreover, aerosol tolerance in rats can be transferred to naive recipients with cells obtained from lymph nodes draining the respiratory tract (12).

However, it remains unclear whether the cervical lymph nodes are crucial for intranasal tolerance induction because application of proteins in the nasal cavity may lead to direct absorption in the blood and systemic spread (13).

Here, we investigate in detail the involvement of the cervical lymph nodes in the induction of tolerance assayed by DTH by removal of the nodes and retransplantation of either nose-draining or peripheral lymph nodes to the sites of the original lymph nodes. Two experimental systems were used: 1) the model Ag OVA in a setting in which a read out was performed in the auricle of the ear and 2) the Ag human cartilage gp-39 (HC gp-39), a candidate autoantigen in RA (14), in a set up in which a DTH response was monitored in the footpad. It was consistently demonstrated that the induction of immunological tolerance is fully dependent on the presence of only those lymph nodes that are originally localized in the nose-draining region.

Materials and Methods

Mice

Six- to eight-week-old female BALB/c mice were obtained from the Neth-erlands Cancer Institute (Amsterdam) (experiments with OVA) or from Charles River (Sulzfeld, Germany) (experiments with HC gp-39) and kept under standard laboratory conditions.

Ag trafficking after intranasal administration

Fifty micrograms of tetramethyl rhodamine isothiocyanate (TRITC) dis-solved in 10 μl 1% DMSO/PBS or 100 μg FITC in 10 μl PBS was applied intranasally with the aid of a micropipette. After 24 h, mice were killed and the superficial cervical, facial, internal jugular (Fig. 1), parathymic lymph nodes, and spleens were removed and frozen in liquid nitrogen. Seven-micrometer cryosections were analyzed under a fluorescence microscope (TRITC-treated mice). Lymph node and spleen cell suspensions (FITC-treated mice) were stained with biotinylated M5/114 (rat anti-mouse MHC class II; Ref. 15) and streptavidin-phycoerythrin (Jackson Immuno Research, West Grove, PA). Numbers of double positive cells were determined by FACS analysis (FACScan; Becton Dickinson, Mountain View, Ca).

Surgical removal of nose-draining lymph nodes

Mice were anesthesized with 10 ml/kg body weight fentanyl/fluanison (Hypnorm)/midazolam (Dormicum, Janssen and Cilag, Saunderton, U.K.)

FIGURE 1. Lymph nodes of the head-neck region (modified from Tilney; Ref. 23).

Table I. *Detection of Ag–bearing cells in cervical lymph nodes and spleen, 24 h after application of FITC intranasally[a]*

	Total Number of FITC+/MHC II+ Cells ($\times 10^5$)		
	Expt. 1	Expt. 2	Expt. 3
Internal jugular lymph node	1.46	0.86	1.50
Superficial cervical lymph node	2.00	5.12	2.36
Facial lymph node	0.18	0.45	0.29
Spleen	9.60	12.3	15.6

[a] FITC was applied intranasally and after 24 h, spleen, internal jugular, superficial cervical, and facial lymph odes were collected. Cell suspensions were prepared and pooled from three mice, 1×10^6 cells were stained for MHC class II expression and 50,000 events were counted by FACS. Numbers represent the absolute number of positive cells in the total organ suspension per mouse from three different experiments.

in sterile water in a 1:1:2 (Hypnorm:Dormicum:water) ratio. A small incision was made in the skin overlying the mandibular glands. With the aid of a surgery microscope, the superficial cervical and/or facial and/or internal jugular lymph nodes were removed and the incision was closed with four to five stitches. At the end of each experiment, the mice were killed and each mouse was examined carefully for regenerated lymph nodes or nodes possibly left behind.

Transplantation of peripheral lymph nodes to the site of the paratracheal nodes

Donor popliteal, inguinal, brachial, and axillary lymph nodes were aseptically collected from naive mice and kept in sterile RPMI 1640 on ice. Recipients were anesthesized with Hypnorm/Dormicum as described above. All cervical nodes were surgically removed. At the internal jugular lymph node sites, a peripheral or a superficial cervical lymph node was placed, one on each side of the trachea. Three weeks after transplantion, intranasal OVA or HC gp-39 administration followed by a DTH-inducing protocol was performed as described below. After the experiment, all mice received rhodamin intranasally to check the presence of afferent lymphatics and in each mouse, the macroscopic appearance and microscopic composition of the transplanted lymph nodes was determined. For the latter, immunohistochemistry was performed using the mAbs MECA 367 (rat anti mouse-MAdCAM; Ref. 16), MECA 79 (rat anti mouse L-selectin ligand Ref. 17), 145-2C11 (hamster anti mouse-CD3; Ref. 18), and 6B2 (rat anti mouse-B cell; Ref. 19).

Induction of tolerance and DTH

Experiments with OVA. Mice received 100 μg OVA/10 μl PBS intranasally with the aid of a micropipette. This was repeated on 3 consecutive days. Control mice received PBS intranasally. The next day after the last intranasal treatment, mice were injected with 100 μg OVA/25 μl PBS/25 μl IFA in the tail base. Five days later, a challenge was given by injecting 10 μg OVA/20 μl PBS in the auricle of both ears, after determining the initial thickness of the ear with an engineer's micrometer (Mititoyo, Tokyo, Japan). The increase in ear thickness was measured 24 and 48 h later.
Experiments with HC gp-39. Mice received 30 μg of HC gp-39/10 μl saline on days 15, 10, and 5 before immunization. Intranasal application was performed under light isoflurane anesthesia with the use of a Hamilton microsyringe adapted to a PT45 microconduit. Animals were immunized with 50 μg of HC gp-39/50 μl PBS/50 μl IFA at two sites on the upper part of the chest region on day 0, and challenged on day 7 in the left footpad with a 50 μl volume of 1 mg/ml HC gp-39 in 1 mg/ml alum; the right footpad was injected with 50 μl of vehiculum and served as a control. The DTH response was monitored 24 h later. A mean percentage-specific paw swelling was determined by measuring the increase in footpad thickness of the left hind footpad compared with the right hind footpad (swelling left (mm) − swelling right (mm)/swelling right (mm) $\times$ 100%), using an in-house designed micrometer.

Results

Intranasally administered Ags localize in both cervical lymph nodes and spleen

To study the trafficking of Ag after intranasal administration, the fluorescent dyes FITC or TRITC were applied onto the nasal mu-

cosa. After 2–24 h, the dissemination of the fluorescent dyes was examined by fluorescence microscopy of the various lymph nodes of the head-neck region (Fig. 1) and of the spleen. Fluorescence was found localized in cells with dendritic morphology present in the subcapsular sinus of the lymph nodes 2–4 h after application. Over time, these cells localized in the paracortical, T cell-dependent areas of the lymph nodes. When the various lymph nodes were compared, substantial numbers of fluorescent cells were found in both the superficial cervical and internal jugular lymph nodes. Only very few cells could be detected in sections of other lymph nodes, draining the head-neck region such as parathymic, mediastinal, and facial lymph nodes. In the spleen, very few positive cells were observed in tissue sections (data not shown). By FACS analysis of organ suspensions, these differences in localization could be confirmed and quantified (Table I). In the superficial cervical nodes about 10 times more cells localized than in the facial lymph nodes. Substantial numbers of cells could also be found in the internal jugular nodes. Strikingly, the absolute number of Ag-bearing cells was highest in the spleen (Table I).

It was furthermore demonstrated that all cells bearing fluorescent dye expressed high levels of MHC class II. Their localization in T cell-dependent areas together with their morphology suggests that these cells belong to the population of dendritic APCs.

Surgical removal of nose-draining lymph nodes prevents the induction of intranasal tolerance

To study the significance of the draining lymph nodes in tolerance induction, all or combinations of the nose-draining lymph nodes were surgically removed. One week after removal of the lymph nodes, intranasal tolerance induction was performed by insertion of OVA into the nose. As shown in Fig. 2, removal of the three types of lymph nodes completely prevented the induction of tolerance; the development and kinetics of the DTH response in the operated mice were completely parallel to that in sham-operated control mice. Removing both superficial cervical and internal jugular nodes, but leaving the facial lymph nodes in their place, prevented tolerance induction (Fig. 3). However, by leaving either the superficial cervical lymph nodes or the internal jugular lymph nodes intact, and removing the other nodes, tolerance induction could still be established (Fig. 3). In concordance with the highest localization of Ag in these nodes, it was demonstrated that the superficial cervical and internal jugular lymph nodes are crucial for tolerance induction.
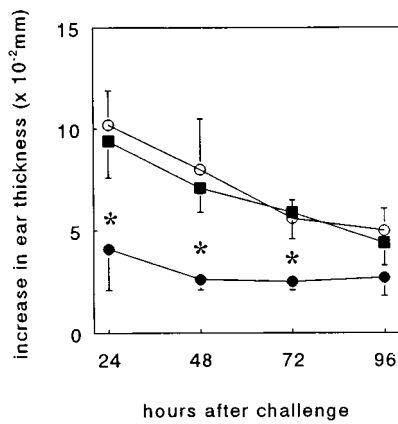
FIGURE 2. Surgical removal of three types of cervical lymph nodes prevents the induction of intranasal tolerance. Mice were either sham-operated (filled dots) or operated to remove cervical lymph nodes (filled squares). After 1 wk, mice were treated intranasally with PBS (open dots) or OVA (filled dots, filled squares), followed by a DTH-provoking protocol. Increase in ear thickness was measured 24–96 h after ear challenge. Each point represents the mean increase in ear thickness of 5 mice $\pm$ SD. *, Significant difference between sham-operated, intranasally OVA-treated mice and both other groups (with at least $p < 0.002$).

intranasal treatment	ND lymph nodes	transplanted SC	transplanted PLN	Increase in ear thickness ($\times 10^{-2}$ mm)
a PBS	+	--	--	
b OVA	+	--	--	**
c OVA	--	--	--	
d OVA	--	+	--	*
e OVA	--	--	+	

FIGURE 4. A peripheral lymph node at a nose-draining site is not able to effectuate intranasal tolerance: studies with OVA. Mice were either sham-operated (*lane b*) or operated to remove nose-draining lymph nodes (*lanes c–e*) after which either superficial cervical lymph nodes (*lane d*) or peripheral lymph nodes (*lane e*) were transplanted to the internal jugular site. After 3 wk, mice were treated intranasally with PBS (*lane a*) or OVA (*lanes b–e*) followed by a DTH-inducing protocol. Each bar represents the mean increase in ear thickness of four to six mice $\pm$ SD, 24 h after ear challenge. ND, nose draining; SC, superficial cervical; PLN, peripheral lymph node. +, Presence of lymph nodes; *, significant difference with *lanes a–c*, and *e* (with at least $p < 0.01$); **, significant difference with *lanes a, c*, and *e* (with at least $p < 0.001$).

Transplantation of peripheral lymph nodes to mucosal sites cannot restore the capacity to induce intranasal tolerance

Removal of the superficial and internal jugular lymph nodes, but not of the facial lymph nodes, led to impairment of tolerance induction. This could be related to the fact that the latter showed only limited drainage of the nasal mucosa. Therefore, we addressed the question of whether exchange of these nose-draining lymph nodes with any nonmucosa-associated lymph nodes would be sufficient to sustain tolerance induction, or whether the cervical lymph nodes were unique in that respect. Thereto, the superficial cervical, the internal jugular nodes, and the facial lymph nodes were removed and the peripheral lymph nodes were transplanted at the site of the internal jugular lymph nodes. Internal control served animals that had their lymph nodes removed and in which the internal jugular nodes were exchanged with the superficial cervical lymph nodes. As established previously, restoration of afferent lymphatics and blood supply was accomplished within 1 wk (20). Three weeks
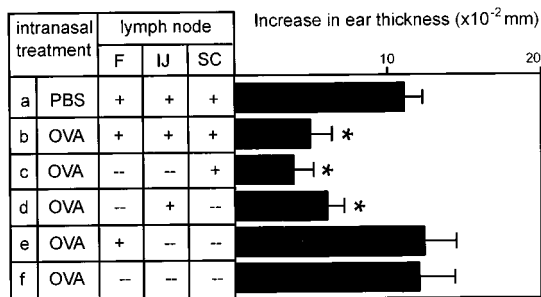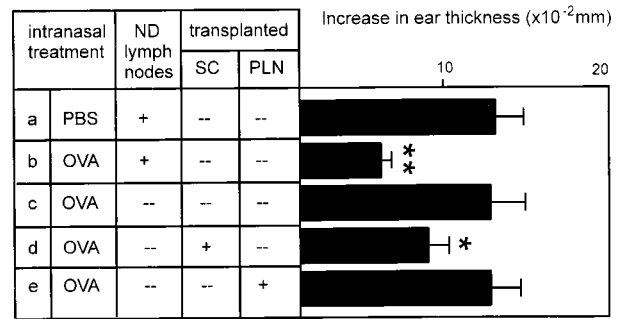
after the operation, the animals were tested for their capacity to become tolerant after application of OVA in the nose. Only those mice that had received the superficial cervical lymph nodes, but not mice which had peripheral axillary nodes transplanted, showed suppression of ear swelling in the DTH assay (Fig. 4). Immediately after the DTH measurement, animals received TRITC in the nose to control for efficient drainage from the nose. In all animals, fully functional transplanted lymph nodes were found as inferred from the presence of TRITC-positive cells (Fig. 5) and normal localization of T and B cells as determined by immunohistochemistry (data not shown).

To confirm these data and to establish the potential relevance of nose-draining lymph nodes in the induction of immunological tolerance in a setting related to autoimmunity, a second experiment was performed with HC gp-39, a candidate autoantigen in RA. Moreover, this time the immunological challenge was performed

intranasal treatment	lymph node F	lymph node IJ	lymph node SC	Increase in ear thickness ($\times 10^{-2}$ mm)
a PBS	+	+	+	
b OVA	+	+	+	*
c OVA	--	--	+	*
d OVA	--	+	--	*
e OVA	+	--	--	
f OVA	--	--	--	

FIGURE 3. Both superficial cervical and internal jugular lymph nodes can effectuate intranasal tolerance. Mice were either sham-operated (*lane b*) or operated to remove internal jugular and facial lymph nodes (*lane c*), superficial cervical and facial lymph nodes (*lane d*), superficial cervical and internal jugular lymph nodes (*lane e*), or all lymph nodes (*lane f*). After 1 wk, mice were intranasally treated with PBS (*lane a*) or OVA (*lanes b–f*) followed by a DTH-inducing protocol. Each bar represents the mean increase in ear thickness of four to five mice $\pm$ SD, 24 h after ear challenge. SC, superficial cervical; IJ, internal jugular; F, facial. +, Presence of lymph node; *, significant difference from (*lane a*) (with at least $p < 0.001$).

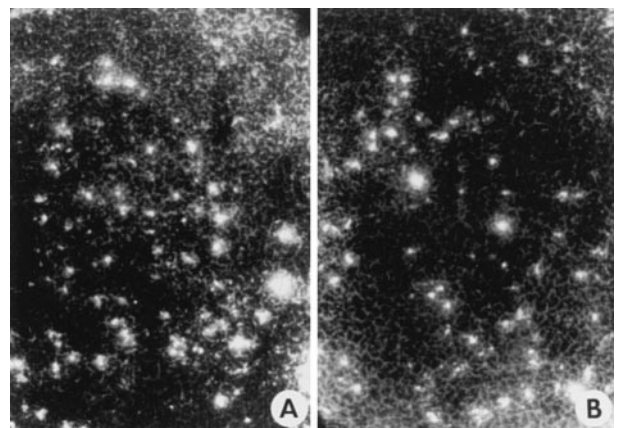**FIGURE 5.** Afferent lymphatics in transplanted lymph nodes are sufficiently restored to allow Ag-bearing cells to enter. Mice that had superficial cervical (*lane A*) or peripheral lymph nodes (*lane B*) transplanted at the internal jugular site were treated with TRITC intranasally. Twenty-four hours later, the transplanted lymph nodes were removed and analyzed under a fluorescence microscope ($\times 20$ objective).
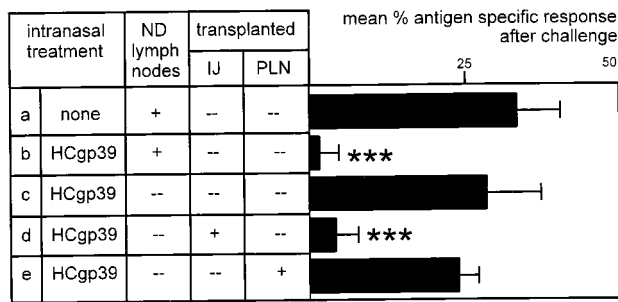
	intranasal treatment	ND lymph nodes	transplanted		mean % antigen specific response after challenge
			IJ	PLN	
a	none	+	--	--	
b	HCgp39	+	--	--	***
c	HCgp39	--	--	--	
d	HCgp39	--	+	--	***
e	HCgp39	--	--	+	

FIGURE 6. A peripheral lymph node at a nose-draining site is not able to effectuate intranasal tolerance: studies with HC gp-39, a candidate autoantigen in RA. Mice were sham-operated (*lane b*) or operated to remove all nose-draining lymph nodes (*lanes c–e*) after which either internal jugular lymph nodes (*lane d*) or popliteal lymph nodes (*lane e*) were transplanted to the internal jugular site. After 3 wk, mice were intranasally treated with HC gp-39 (*lanes b–e*) or not tolerized (*lane a*) followed by immunization and DTH by footpad challenge. Each bar represents a mean percentage increase of footpad swelling as compared with the vehicle challenged footpad ± SD, 24 h after challenge. ND, nose draining; IJ, internal jugular; PLN, popliteal lymph node. +, Presence of lymph nodes; −, absence of lymph node (s); ***, significant difference with *lanes a, c,* and *e* (with at least $p < 0.005$).

in the footpad, avoiding potential influences of manipulation performed in the head-neck region as a result of surgery 3 wk earlier. A strong DTH reaction (34.7% specific swelling) was induced in animals immunized and challenged with HC gp-39. Animals that were sham-operated, leaving the nose-draining lymph nodes intact before the induction of tolerance, did not respond in the DTH assay; mice were fully tolerant to the HC gp-39 protein. Removal of the superficial cervical and internal jugular lymph nodes abolished tolerance, confirming the role of nose-draining nodes in tolerance induction. In mice with a jugular but not a popliteal transplant, the capacity to induce tolerance was restored (Fig. 6). Again, after measurement of the DTH response, mice were visually inspected for vascularization of the transplanted lymph node and restoration of draining lymph vessels. Data from two animals were excluded; in one animal the transplanted lymph node was infected and in one other animal a macerated-lymph node transplant was found, probably the result of nonvascularization.

Collectively, the above presented data point to unique functional characteristics of nose-draining lymph nodes in the process of tolerance induction.

Discussion

Intranasal administration of Ag has been put forward as an effective means to induce Ag-specific immunological tolerance. In case of autoimmune diseases, this natural and powerful mechanism can be exploited therapeutically. Insight into the precise mechanisms involved in tolerance induction via the nasal mucosa may be instrumental in the further optimization of this process. In this study, we have used the model Ag OVA and a candidate autoantigen in RA, HC gp-39 (14), to study the role of nose-draining lymph nodes in the induction of immunological tolerance.

We have demonstrated that in mice, independent of the Ag and the site of immunological challenge with such Ag, induction of tolerance via the nasal mucosa is strictly dependent on the presence of the superficial cervical or internal jugular lymph nodes. Moreover, these lymph nodes contain crucial intrinsic characteristics, because replacement of these lymph nodes with nodes from peripheral sites does not lead to tolerance induction. The efficiency of tolerance induction can be concluded from the fact that only one set of lymph nodes is sufficient. In fact, in the onset of our series of lymph node removal experiments, occasionally an animal became tolerant in spite of assumed complete removal of the lymph nodes. Upon inspection, these animals always showed incomplete removal, demonstrating that a single lymph node of either superficial cervical or internal jugular type was nevertheless sufficient to induce tolerance.

When lymph nodes are transplanted from one site to another, the disruption of blood supply and lymphatics causes major changes in the organization of the lymph node that are re-established as soon as the lymphatics and blood vessels grow in. This occurs within 7 days (20). The majority of hematological cells, macrophages, dendritic cells, and lymphocytes will be replaced after this time, indicating that the intrinsic properties of the lymph node, as found in our tolerance experiments, must predominantly reside in stromal elements such as reticular cells, extracellular matrix components, or endothelial cells leading to specific immigration of cells from the blood.

Previously, we showed that tolerance induction via the nose involves active T cell-mediated suppression (9, 10). Hereto efficient Ag presentation has to take place by APCs that enter the lymph nodes via afferent lymphatics. No differences between localization of Ag-bearing cells in the paracortical areas of transplanted mucosal or peripheral lymph nodes were found (data not shown). Therefore, differences in Ag presentation must also lie at the level of the lymph node microenvironment, which may influence the regulation and expression of accessory molecules on the incoming dendritic cells. In addition, differences at the level of the immigrating T cells may also be important. In this respect, it is interesting to note that the mucosal superficial cervical and the internal jugular lymph nodes draining the nasal mucosa express the mucosal addressin MAdCAM-1 on their high endothelial venules. This adhesion molecule is absent in the facial lymph nodes and in the transplanted peripheral lymph nodes. After transplantation of peripheral lymph node, MAdCAM-1 expression was never found on high endothelial venules, but it was readily expressed on the transplanted superficial cervical nodes (Fig. 7). This is in accordance with our previous findings in which we established that the expression of addressins on high endothelial venules is determined perinatally and cannot be changed after ectopic transplantation of lymph nodes in adult life (20). These findings imply that differences in lymphocyte homing behavior and subsequent composition of lymphocyte population can exist between lymph nodes that may underlie the observed divergence in tolerance induction.

In addition to differential recruitment of lymphocytes, differences in macrophage function between peripheral and mucosal lymph nodes have been demonstrated. Macrophages in mucosal lymph nodes have a significantly lower activity of the enzyme that converts the prohormone dehydroepiandrosterone-(DHEA-) sulfate into the active form DHEA (21). DHEA has immunomodulatory activities (22) that might consequently result in differences in the microenviroment of lymph nodes. Preliminary experiments in which macrophages were depleted from the nose-draining lymph nodes by toxic liposomes did not show an effect of macrophages in tolerance induction. It must be noted that using this method, macrophages residing in the paracortex are not depleted. Considering their strategic position in the paracortical area in which T cells and dendritic cells interact, these cells could play an important role.

As seen in the localization studies, substantial numbers of Ag-bearing cells localize in the spleen after the application of Ag in the nose. It is not clear whether this leads to any substantial antigenic stimulation of T cells in the spleen. Removal of the nose-draining lymph nodes did not lead to enhanced localization of
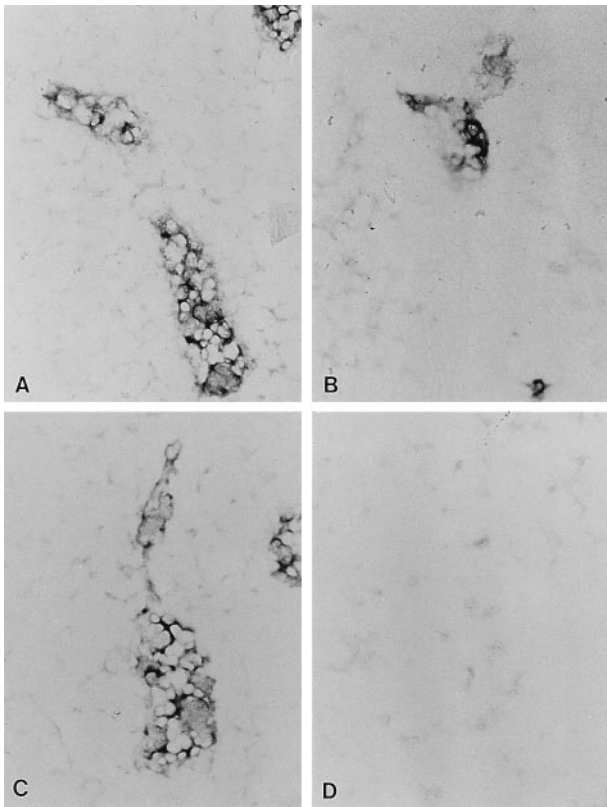
FIGURE 7. Expression of vascular addressins on transplanted lymph nodes. Four weeks after transplantation of either a superficial cervical lymph node or a peripheral lymph node to the position of the cervical lymph node, consecutive sections of the lymph nodes were stained for the expression of the L-selectin ligand MECA-97 (*A* and *C*), and MAdCAM-1 (*B* and *D*). *A* and *B* are consecutive sections of a transplanted cervical lymph node showing partial expression of MAdCAM-1 on high endothelial venules (*B*). In transplanted peripheral lymph nodes, no MAdCAM-1 expression is observed (*D*). Magnification, ×20 objective.

Ag-bearing cells in the spleen as determined by FACS (data not shown), or to the induction of tolerance. This argues against a specific role of the APCs from the mucosal region in tolerance induction and emphasizes the importance of the lymph node microenvironment.

In our experiments, we were not able to show a major effect of tolerance induction on the levels of specific Igs, although a shift toward Th2 isotypes was observed (data not shown and Ref. 10). This could mean that in this model T cell tolerance is more readily induced at the level of DTH responses than T help for B cell activation. The Ag dose and efficiency of uptake may be crucial as suggested by Viney et al. (24), who found that by in vivo increasing the number of DC, tolerance could be induced with lower amounts of Ag.

Taken together, the results show an intrinsic capacity of cervical lymph nodes to induce immunological tolerance. Factors determining this capacity could lie at the level of lymphocyte entrance and retention, but must be based on differences of stromal cells that form the basic structure of the node. It will be of great importance to study the nature of these differences to be able to use this for therapeutic applications in conditions in which tolerance induction can be extremely helpful, as in autoimmunity and allergies. As such, it is of interest that the basic rules defined in this paper, the crucial role of nose-draining lymph nodes in the induction of immunological tolerance, hold true for a model Ag (OVA) as well as for HC gp-39, a candidate autoantigen in the autoimmune disease

RA. Intranasal administration of HC gp-39 has been shown not only to induce immunological nonresponsiveness to subsequent immunization with this Ag, but also to suppress HC gp-39-induced arthritis in BALB/c mice (14). Thus, the fact that nose-draining lymph nodes are essential in the process of tolerance induction may provide further clues for effectively treating human autoimmune conditions with nasally administered autoantigens.

References

1. Mowat, A. M. 1987. The regulation of immune responses to dietary protein antigens. *Immunol. Today 8:93.*
2. Weiner, H. L., A. Friedman, A. Miller, S. J. Khoury, A. Al-Sabbagh, L. M. Santos, M. H. Sayegh, R. B. Nussenblatt, D. E. Trentham, and D. A. Hafler. 1994. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu. Rev. Immunol. 12:809.*
3. Hoyne, G. F., R. E. O'Hehir, D. C. Wraith, W. R. Thomas, and J. R. Lamb. 1993. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J. Exp. Med. 178:1783.*
4. McMenamin, C., C. Pimm, M. McKersey, and P. G. Holt. 1994. Regulation of IgE responses to inhaled antigen in mice by antigen-specific γδ T cells. *Science 265: 1869.*
5. Tian, J., M. A. Atkinson, M. Clare-Salzer, A. Herschenfeld, T. Forsthuber, P. V. Lehmann, and D. L. Kaufman. 1996. Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin dependent diabetes. *J. Exp. Med. 183:1561.*
6. Dick, A. D., Y. F. Cheng, J. Liversidge, and J. V. Forrester. 1994. Intranasal administration of retinal antigens suppresses retinal suppresses retinal antigen-induced experimental autoimmune uveoretinitis. *Immunology 82:625.*
7. Weiner, H. L. 1997. Oral tolerance: immune mechanisms and treatment of autoimmune diseases. *Immunol. Today 18:335.*
8. Miller, A., Z. J. Zhang, R. A. Sobel, A. Al-Sabbagh, and H. L. Weiner. 1993. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. VI. Suppression of adoptively transferred disease and differential effects of oral vs. intravenous administration. *J. Neuroimmunol. 46:73.*
9. Van Halteren, A. G. S., M. J. F. Van der Cammen, D. Cooper, H. F. J. Savelkoul, G. Kraal, and P. G. Holt. 1997. Regulation of antigen-specific IgE, IgG1 and mast cell responses to ingested allergen by mucosal tolerance induction. *J. Immunol. 159: 3009.*
10. Wolvers, D. A. W., M. J. F. Van der Cammen, and G. Kraal. 1997. Mucosal tolerance is associated with, but independent of, upregulation of Th2 responses. *Immunology 92:328.*
11. Hoyne, G. F., B. A. Askonas, C. Hetzel, W. R. Thomas, and J. R. Lamb. 1996. Regulation of house dust mite responses by intranasally administered peptide: transient activation of CD4⁺ T cells precedes the development of tolerance in vivo. *Immunol. Invest. 8:335.*
12. Sedgwick, J. D., and P. G. Holt. 1985. Induction of IgE-secreting cells and IgE isotype specific suppressor T cells in the respiratory lymph nodes of rats in response to antigen inhalation. *Cell. Immunol. 94:182.*
13. Swarbrick, J., and J. C. Boylon. 1994. Nasal drug delivery: trends and perspectives. *Encycl. Pharm. Techn. 10:191.*
14. Verheijden, G. F. M., A. W. M. Rijnders, E. Bos, C. J. J. Coenen-de Roo, C. J. van Staveren, A. M. M. Miltenburg, J. H. Meijerink, J. H., D. Elewaut, F. de Keyser, E. Veys, and A. M. H. Boots. 1997. Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis. *Arthritis Rheum. 40:1115.*
15. Bhattacharya, A., M. E. Dorf, and T. A. Springer. 1981. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J. Immunol. 127:2488.*
16. Streeter, P. R., E. L. Berg, B. T. N. Rouse, R. F. Bargatze, and E. C. Butcher. 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature 331:41.*
17. Streeter, P. R., B. T. N. Rouse, and E. C. Butcher. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol. 107:1853.*
18. Leo, O., M. Foo, D. Sachs, L. Samelson, and J. Bluestone. 1987. Identification of a monoclonal antibody specific for murine T3 polypeptide. *Proc. Natl. Acad. Sci. USA 84:1374.*
19. Coffman, R. L. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunol. Rev. 69:5.*
20. Mebius, R. E., J. Brevé, G. Kraal, and P. R. Streeter. 1993. Developmental regulation of vascular addressin expression: a possible role for site-associated environments. *Int. Immunol. 5:443.*
21. Daynes, R. A., B. A. Araneo, T. A. Dowell, K. Huang, and D. Dudley. 1990. Regulation of murine lymphokine production in vivo. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell functions. *J. Exp. Med. 171:979.*
22. Daynes, R. A., D. J. Dudley, and B. A. Araneo. 1990. Regulation of murine lymphokine production in vivo. II. Dehydroepiandrosterone is a natural enhancer of interleukin-2 synthesis by helper T cells. *Eur. J. Immunol. 20:793.*
23. Tilney, N. L. 1971. Patterns of lymphatic drainage in the adult laboratory rat. *J. Anat. 109:369.*
24. Viney, J. L., A. M. Mowat, J. M. Omalley, E. Williamson, N. A. Fanger. Expanding dendritic cells in vivo enhances the induction of oral tolerance. *J. Immunol. 160:5815.*