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Review article

Lymphocyte migration studies

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Abstract – For maintenance of immunity and tolerance, the organs and tissues of the organism are connected by migrating lymphoid cells. Understanding lymphocyte migration is essential for many disorders and diseases – especially in the mucosa-lined organs. Detailed analyses of migrating lymphocytes have been performed in many species, especially in laboratory animals. However, important experiments in lymphocyte migration have been carried out in large animals, for example sheep, cattle and pigs. These species allow experimental procedures like in situ-organ labelling, lymphocyte retransfusion studies or lymph vessel cannulations. Such studies have made an important contribution to the understanding of the overall principles of lymphocyte migration especially in the mucosal immune system. Major results on the specific migration of naïve and memory T cells through lymphoid organs, the re-distribution of γ/δ T cells in the intestinal immune system and the emigration of newly produced B cells from the ileal Peyer's patches have been obtained in large animals. Since there are growing numbers of markers for large animals, and molecular biology methods are available in these species, experiments in large animals will be an essential tool for the understanding of lymphocyte migration especially in mucosal organs.

lymphocyte recirculation / cell transfer / lymph cannulation / adhesion molecules / Peyer's patches

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1. INTRODUCTION

Lymphocyte migration is an important area of immunological research. Antigen-specific lymphocytes are located in almost every mucosal and non-mucosal tissue compartment of the mammalian organism. The continuous cell traffic between different sites connects these compartments as well as representing different stations during B and T cell development: in general, naïve lymphocytes recirculate through secondary lymphoid organs where priming occurs, followed by homing to effector sites. Active migration of antigen specific lymphocytes additionally increases their chance to encounter specific antigens. This review focuses on migration routes in the gastrointestinal and respiratory tract, and gives some additional information on lymphocyte migration in the mammary gland and uterus. Studies in large animal species like sheep and pigs are important to detect the mechanisms of lymphocyte migration and their impact on immunity. These studies are superadditive to experiments in rodents – since large animal experiments allow multiple blood samples to be taken over a longer period of time. If the animals are well trained, this experimental approach is less stress inducing than with multiple blood samples in for example rats. Furthermore, lymph vessel cannulation techniques give access to areas of the body in sheep, cattle and pigs such as the intestinal wall, the mesenteric lymph nodes or various peripheral lymph node groups. These areas can not be experimentally lymph-drained in mice and rats without serious problems. In these species, thoracic duct cannulations can be performed – however the animals have to be kept on a running wheel (mice) [89] or within a metabolic cage (rats) [98] – both methods obviously induce stress that has implications on lymphocyte migration as well as the underlying immune reaction.

2. LYMPHOCYTE MIGRATION ROUTES IN MUCOSAL TISSUE COMPARTMENTS

2.1. Gastrointestinal tract

The gastrointestinal tract is one of the main entry sites for pathogens. In addition to lymphocytes in draining lymph nodes, the lamina propria and the epithelium, the intestinal tract is equipped with specific lymphoid tissue structures. This gut-associated lymphoid tissue (GALT) consists of lymphoid follicles within the gut wall with overlying M cells – epithelial cells specialised in luminal antigen uptake – in morphologically distinct "dome"-like villi. The Peyer's patches (PP) instead of the draining lymph nodes are thought to be the main inductive site for intestinal immune responses. Blood lymphocytes enter the PP via high endothelial venules (HEV). Here, antigen encounter drives the development of effector and memory lymphocytes, which then leave the PP via afferent intestinal lymph vessels [71]. Five to fifteen percent of lymphocytes leaving the gut have been newly formed [78]. Further expansion and maturation of the PP-derived lymphocytes then occurs in the mesenteric lymph nodes [81]. Alternatively, the mesenteric lymph nodes may serve as primary inductive sites for lymphocytes entering directly from the bloodstream. Primed lymphocytes leave the mesenteric lymph nodes via efferent lymph vessels, access the bloodstream, and home to effector sites, i.e. the intestinal lamina propria, the intestinal epithelium, or to other peripheral lymph nodes. Additionally, some peripheral lymph node blasts also home to the gut [58]. However, it has been demonstrated that gut-derived lymphocytes preferentially migrate back to the GALT and to mesenteric lymph nodes [56]. While lymphocytes enter PP and mesenteric lymph nodes via HEV, entry into the lamina propria occurs through postcapillary venules located in the crypt regions of the intestinal

wall [79]. Lymphocyte proliferation in pigs can occur in the mesenteric lymph nodes, in all compartments of the PP (i.e. follicles, dome areas, and interfollicular regions) [79], but also in the lamina propria or in the intestinal epithelium [75, 80], and mitotic figures indicating proliferation have also been detected in cells migrating in intestinal lymph [76].

In addition to these general principles more detailed analyses of the gastrointestinal immune system have revealed further compartment-specific or species-specific aspects. Thus, jejunal and ileal PP differ in lymphocyte homing patterns [74]. Cell traffic through jejunal PP is generally higher with few lymphocytes homing to ileal PP [56]. Furthermore, instead of preferentially homing back to intestinal sites, B and T cells emigrating from lamb ileal PP indicate no preference to extravasate into gut tissues [72].

A specific developmental mechanism was also suggested for intraepithelial lymphocytes (IEL) [29]. However, the so-called extrathymic pathway for intraepithelial T cell ontogeny does not seem to be relevant under normal circumstances [28]. IEL can migrate into the epithelium from different sources, e.g. the spleen [51], but they do not emigrate again.

An important species-specific peculiarity in the pig is the "inverted" architecture of their lymph nodes. In contrast to sheep where lymphocytes emigrate from lymph nodes exclusively through efferent lymph vessels [18], resulting in high lymphocyte numbers in efferent lymph – in comparison to the afferent sheep lymph, porcine lymphocytes leave the lymph nodes via postcapillary venules [9]. Pig lymphocytes migrating from the PP thus pass through the capillary bed of the portal vein in the liver. The functional relevance of this characteristic is still largely unknown.

2.2. Respiratory tract

The lung is the second largest mucosal surface of the mammalian organism, only exceeded in size by the gastrointestinal tract. The lymphocyte content of the lung is similar to that of discrete PP in pigs, calves and lambs [58]. Like the intestinal immune system, the respiratory tract immune system includes lymphocytes in interstitial compartments and draining lymph nodes, as well as some intraepithelial cells. Analogous structures to the PP of the GALT also exist, as bronchus-associated lymphoid tissue (BALT) which is located at the bifurcations of larger bronchi [60]. However, in contrast to GALT, BALT is not constitutive but rather seems to develop after antigenic stimulation. In addition, there are species-specific differences. Thus, in contrast to germ-free rats, germ-free pigs lack BALT. BALT is rare in normal pigs (33%), but more frequently seen in sheep [6, 59]. Furthermore, BALT has a more diffuse morphology than GALT, and consists mainly of single follicles. A distinctive feature of the lung is the presence of a large intravascular pool of lymphocytes associated with blood vessel walls, which is easily mobilisable. About 1.5×10^9 lymphocytes can be collected continuously over a period of 4 h when one pig lung is perfused with cell free medium [57]. Another characteristic site of the respiratory tract is the bronchoalveolar space, but only about 10% of cells present in this compartment are lymphocytes [64].

These different compartments are connected by a large number of migration pathways [61]. Thus, a compartmentalised response can be mounted by lymphocytes that enter BALT from the intravascular pool via HEV, and migrate to regional lymph nodes through afferent lymph vessels after antigen encounter. A more frequent alternative is that primary antigen handling occurs in the regional lymph nodes by lymphocytes that have entered directly from the blood stream, or by lymphocytes that have migrated into the lymph nodes from the interstitium. Lymphocytes can enter the bronchoalveolar space from the BALT or from the interstitial tissue. From there, many cells are probably lost by the "mucociliary escalator", which

transports luminal contents through the trachea to the pharynx region where they are swallowed. However, a small proportion of lymphocytes in the bronchoalveolar space can also move back through the epithelium into interstitial tissue. Indeed, entry of luminal CD8+ T cells into the BALT through M cells has been demonstrated in pigs [62]. In general, lung lymphoblasts preferentially migrate back to the lungs [61].

Not much is known about lymphocyte turnover rates in the respiratory tract. After injecting labelled blood lymphocytes i.v. into pigs, about 10% of them can be detected in the lungs 30 min later [58], most of them in the intravascular pool. After instillation into the bronchoalveolar space, labelled lymphocytes can be found in the interstitium and regional lymph nodes a few hours later [62].

Preferential migration to the respiratory tract has also been demonstrated for gut-derived lymphocytes. For example, oral application of the lung pathogenic bacterium *Actinobacillus pleuropneumoniae* in young pigs resulted in significantly increased numbers of both T cells and plasma cells in the bronchoalveolar space [19]. Likewise, lymphocytes from porcine mesenteric lymph nodes migrate to the lung [50]. In sheep, the distribution of IgA positive plasma cells similarly indicates that the IgA+ cells in efferent mediastinal lymph originate from intestinal mucosal sites rather than from the respiratory tract [87]. This clinically important principle called the "integrated mucosal immune system" allows protection against respiratory disease by oral vaccination. However, the integration seems to be only one-directional, since the migration rate of lymphocytes from efferent lymphatics of the lung to mesenteric lymph nodes is significantly lower than the migration to pulmonary or to other peripheral nodes [88].

2.3. Tonsils

Located in the upper aero-digestive tract, the tonsils present a first line barrier against both respiratory and intestinal antigens and are thus an important part of the integrated mucosal immune system. Though less well defined in their internal structure, they are morphologically similar to PP, with follicles, M cells and HEV. Lymphocyte production of the tonsils per gram tissue equals that of peripheral lymph nodes [55].

Naïve blood lymphocytes enter the tonsils [52] and, after priming, home preferentially to local, that is cervical lymph nodes, but also to other peripheral and mesenteric lymph nodes. In contrast, homing to PP or the spleen is rare. In young pigs, 1% of all labelled blood lymphocytes enter the tonsils – thus lymphocyte entry into the tonsils is relatively higher than entry into PP [58]. Additionally, migration of splenic or lymph node derived lymphocytes into the tonsils has been demonstrated. Interestingly, lymphocyte migration patterns can also vary between individual tonsils, probably due to a distinct expression of adhesion molecules and chemokines. Thus the proportion of T cells and B cells differ between palatine and pharyngeal tonsils in pigs [16].

2.4. Mammary gland

The immune system of the mammary gland consists of immune cells in the tissue lamina propria, in mammary secretions, and in the draining lymph nodes. Like the respiratory tract, the mammary gland is part of the "integrated mucosal immune system". For instance, Kortbeek-Jacobs et al. [37] demonstrated preferential accumulation of sensitised cells in the mammary gland after oral immunisation of sows with *E. coli* during lactation. This functionally important "entero-mammary link" mediates immunity of the offspring against antigens present in the maternal digestive tract [84]. Antigen-specific lymphocytes migrate into the interstitial layer of the gland, and then produce sIgA which is secreted into the gland lumen, or even move into the lumen themselves.

Although the majority of IgA plasma cells in the sow's mammary gland is derived

from mesenteric lymph nodes, local immune mechanisms also play a role. In the sow, antigen injection into the glandular tissue during late pregnancy or during lactation results in the appearance of specific antibodies in milk [83], and in increased densities of B and T cells in the lamina propria of the gland [40]. Migration of activated T cells into the gland lumen of sheep can be induced by infusion with lipopolysaccharide (LPS) or with parasite larvae [26]. In general, the distribution of lymphocyte subsets in the mammary gland is strongly influenced by lactation and gestation stage [95, 102]. Furthermore, the lactating mammary gland also seems to specifically influence the T cell subset composition in blood after parturition – an effect which can be abolished by mastectomy [36].

2.5. Uterus

Within the mucosal immune system, the genital tract mucosae play a special role. In the uterus, lymphocytes can be found in the epithelial layer and the interstitial layers, but no defined mucosa-associated lymphoid tissue exists. As in the mammary gland, lymphocyte migration in uterine tissue is greatly influenced by hormonal changes during pregnancy. Furthermore, differences in subset composition and migration patterns have been demonstrated for tissue areas with or without contact to the conceptus [20, 38, 46].

Unlike respiratory and mammary gland mucosae, the genital tract may not be part of the "integrated mucosal immune system". No preferential migration of mesenteric lymph node blasts to the genital tract mucosa could be demonstrated for male rats [23], and the same seems to apply to the female genital tract [58]. In humans, oral immunisation was not an efficient way to elicit genital antibody responses, whereas rectal or nasal immunisation was more promising [82]. To date, few studies have been performed in farm animals to clarify that matter.

3. LYMPHOCYTE COMPARTMENTS AND METHODS FOR LYMPHOCYTE MIGRATION STUDIES

The blood contains at one given moment about 5% of all lymphoid cells, their half-life in this intravascular compartment is about 30 min [63]. Thus, the blood stream is mainly a transport system for lymphoid cells, and blood lymphocyte counts as well as phenotypes do not represent the immune status of the organism [97]. The number of lymphoid cells in the various organs and lymphoid structures of the body are difficult to estimate. In pigs a very careful analysis was performed: the weight of all lymphoid tissues was determined, lymphocytes counts were performed and the DNA content of single lymphocyte and cell preparations was analysed [49]. In these experiments, the mucosal organs contained about 23% of the total number of 321×10^9 lymphocytes of the pig's body. In more detail, the numbers of intraepithelial and lamina propria lymphocytes were studied in pigs of various ages. Separation of lymphocytes from the small intestinal mucosa of adult pigs resulted in 26.8×10^6 IEL and in 35.2×10^6 lamina propria lymphocytes per gram of fresh tissue [77]. But so far, no well-defined methods have been developed to quantify lymphocyte emigration from the intestinal mucosa. It remains to be clarified to what extent the organised lymphoid structures such as the PP and the mucosa itself contribute to the export of lymphocytes into the organism.

Ex vivo perfusion experiments have been designed to follow the exit of lymphocytes from a whole organ like the spleen or the liver [58]. Another approach is the injection of a labelled cell population and the determination of the labelling index of those cells in the organs. The index then reflects the migration rate of the lymphocytes [8, 58, 92].

Detailed studies of lymphocyte migration require an appropriate labelling system. Migrating lymphocytes have to be detectable among all cells of the blood and

organs – either in cell suspensions or in situ in their tissue location. In early studies the cells were labelled by ^{51}chromium and the radioactivity in samples was determined [21]. Since chromium is released from dying labelled cells and distributed as free ion in blood and organs, the radioactivity measured might not directly reflect the cell distribution in the organism. A better label is fluorescein-isothiocyanate (FITC). The advantage of this label has carefully been demonstrated, and it is possible to study the distribution of cells in their histological situation [8, 10, 100]. Other fluorescence markers have been developed which enable a longer lasting staining profile e.g. PKH-2 or carboxyfluorescein diacetate succinimidyl ester (CFSE) [3, 32, 91]. However, a recent publication stresses that many fluorescent dyes especially affect B cells during migratory processes [48]. Bromodeoxyuridine can be used for labelling of all cells in the S-phase of the cell cycle [99]. A very interesting "labelling" method is the use of syngeneic animal strains. Such studies have been done with inbred strains of pigs, where a polymorphism of the leukocyte common antigen (CD45) was used to detect donor lymphocytes during migration experiments [13].

Its of major importance to select a clearly defined cell population to follow in the migration experiments. Either blood lymphocyte preparations or cell preparations from organs can be used. In rodents, these populations can be labelled and retransfused into syngeneic recipients – as described in an early review [21]. With few exceptions, an inbred approach is not possible in large animals [13]. Therefore large volumes of peripheral blood were used, the lymphocytes were separated, labelled in vitro and retransfused for the migration studies. By using this method, up to 1×10^9 labelled lymphocytes were retransfused in for example pigs [74]. The follow-up of the injected cells is possible even in organs showing very little immigration like the thymus.

A further method for collecting large numbers of lymphocytes for migration studies is the cannulation of lymph vessels draining the intestines [71]. In experiments in sheep, the numbers of emigrating cells and their phenotype during the cannulation period were studied [70]. The major intestinal lymph duct cannulation is also established in the pig [76], however in pigs a mesenteric lymph node resection is necessary since the pig's efferent lymph is poor of cells [7]. Interestingly, lymphocyte migration in intestinal pseudo-afferent lymph differs between lambs and minipigs, with average rates of 800×10^6/h and 30×10^6/h, respectively [71, 92].

An extremely sophisticated experimental approach is the in situ labelling of a defined section of the gut via the arterial and venous vessels with a dye (e.g. FITC) after interruption of the normal circulation. Having performed the labelling, blood circulation is re-established and the migration of the lymphocytes can be followed [54]. An easier method for local labelling is the injection of the dye into a parenchymatous organ like the tonsils, followed by the detection of labelled lymphocytes in other compartments of the body [52].

4. IMPORTANT INTESTINE-RELATED LYMPHOCYTE MIGRATION STUDIES IN LARGE ANIMALS

Not all experiments performed in the mucosal immune system of large animals can be reviewed here. As examples, some experiments may demonstrate the impact of these species in the understanding of immunological mechanisms. Typical lymphocyte migration pathways have been studied in the mucosal immune system in the initial lymphoblast retransfusion experiments in rats [30]. By performing recirculation studies, it was demonstrated that lymphoblast and lymphocytes follow different migration routes. Lymphoblasts preferentially migrate back to mucosal sites [11].

The use of lymphocyte labelling techniques of the ileal PP in sheep and following

the exit of the cells in comparison to cell proliferation in the follicles demonstrated a large number of apoptotic B cell precursors in ileal PP follicles [69]. Based on these observations, a major debate on the role of ileal PP in sheep, cattle and pigs for antigen independent lymphocyte production has been induced [5, 67]. The continuous ileal PP of ruminants has been discussed as a primary lymphoid organ for B cell development equivalent to the Bursa Fabricii in avian species [27]. Some typical features of the ileal PP indicating primary lymphoid organ function have been demonstrated: they are major contributors to the total B cell pool, even in the fetal lamb in the absence of foreign antigen [68]. Lymphocytes in ileal mesenteric lymph nodes of sheep are largely derived from ileal PP [72], and 10–20% of cells leaving the PP areas of the terminal ileum in sheep are dividing cells, indicating a high level of cell proliferation [71]. Although lamb ileal PP produce a large number of lymphocytes, only a few of them leave via afferent lymph vessels, whilst most of them remain in situ or become apoptotic [54]. This might be due to an affinity selection process. As in the thymus, the primary lymphoid organ for T cell production, entry of new progenitor cells into ileal PP closes relatively early, and involution of the organ occurs later in life. Porcine continuous PP may have comparable functions to those in ruminants since they have several functional similarities [5].

A specific migration of cell subsets has been demonstrated in large animals for various types of lymphocytes. B cell transit from PP to the lamina propria in lambs takes about 1 day [54]. Notably, this includes transformation of resting B lymphocytes into plasma cells. Similar observations were made in pigs, where lymphocytes from pseudo-afferent intestinal lymph were detected in all lymphoid organs one day after i.v. retransfusion [74]. Naïve and memory lymphocytes were analysed in sheep and their tissue specific immigration was obvious [41–43]. Based on these experiments, the concept about naïve and memory T cells was clarified. Initial experiments in pigs showed that especially naïve (CD45RC positive) T cells emigrate from the intestinal mucosa. These preliminary results also indicate that those CD45RC positive cells indeed originate largely from the PP instead of the normal lamina propria [4]. Using a combination of fluorescent dye (all lymphocytes) and bromodeoxyuridine (proliferating cells) in intestinal lymph duct cannulated pigs it was possible to demonstrate the preferential location of IgA+ B cells/plasma cell precursors in the lamina propria of these animals [81]. Comparable experiments were performed in rodents much earlier. The advantage of these studies in pigs, however, was the use of plasmoblast-precursors that originated from a migrating cell population – in this case from the intestinal lymph. Thus the experimental setup was closer to the in vivo situation than those experiments that were performed with cell suspensions taken from lymphoid organs such as mesenteric lymph nodes or PP. To obtain these cells, a mechanical separation is necessary, exposing the cells to additional stress.

It is obvious that the distribution of specific cell subsets along the intestinal tract can only be explained by lymphocyte migration. This was demonstrated by CDR3-spectratyping in pigs of various age groups. In older animals, it was detected that single expanded γ/δ T-cell clones are present only in the intestinal wall [34]. The distribution along the gut is possible by the transport mechanisms of the lymph vessels (for exit from a tissue) and the blood (for entry into the tissue). Experiments using lymph duct cannulated pigs revealed that intestinal lymph contains a marked proportion of γ/δ T-cells that were produced in the intestinal wall. After in vitro labelling this cell subset preferentially remigrates into the intestinal wall [93]. Experiments in sheep have also demonstrated the quick recirculation of γ/δ T cells [103, 104]. However, lymphocyte migration studies in the mucosal immune system in large animals so far have not contributed to the understanding of the

Table I. Examples of adhesion molecule-specific antibodies available for sheep, cattle, and pigs.

Marker	Species	Expression	Reactivity	Reference	Example clones
CD44	Porcine	Lymphocyte	Pig	[105]	MAC325
ICAM-1	Porcine	Endothelium	Pig	[90]	19C7
VCAM-1	Porcine	Endothelium	Pig	[90]	10.2C7
E-selectin	Human	Endothelium	Pig	[35]	1.2E6
L-selectin (CD62L)	Ovine	Lymphocyte	Sheep, cattle	[42]	Du1-29
LFA-3	Ovine	Lymphocyte	Sheep	[42]	L180/1
LFA-1	Porcine	Lymphocyte	Pig	[2]	BL1H8
VCAM-1	Human	Endothelium	Sheep, cattle	[42]	HAE2-1
β1 integrin	Ovine	Lymphocyte	Sheep, cattle	[42]	47
α6 integrin	Human	Lymphocyte	Sheep, cattle, pig	[42]	GoH3
β2 integrins (CD18)	Porcine	Lymphocyte	Pig	[33]	MUC76A
MAdCAM-1	Human	Endothelium (mucosal lymph nodes)	Cattle, sheep	[66]	7G11
PNAd (L-selectin ligand)	Murine	Endothelium (peripheral lymph nodes)	Cattle, sheep, pig	[66]	MECA-79

regulation of antigen specific cell migration. The different experimental approaches are not suitable to clearly demonstrate what traffic of cells is necessary for establishing an appropriate immune reaction. If the selected clones of the experiments observed can be later on attributed to specific antigens, the initial step for migration studies using antigen specific lymphocytes can be performed in the mucosal immune system. The development of tetramers of swine SLA class 1 will enable such experiments in the future [24].

5. REGULATION OF LYMPHOCYTE MIGRATION

The complex cell traffic network between different mucosal sites is controlled by a number of surface proteins expressed by lymphocytes on the one hand (e.g. integrins), and by endothelial cells on the other. The term "homing" signifies the arrival of a lymphocyte in the tissue of a lymphoid or non-lymphoid organ, usually from the bloodstream. Adhesion molecules expressed on a specific lymphocyte thus determine in which tissue compartment the cell ends up. A selection of antibodies against bovine, ovine and porcine adhesion molecules is given in Table I. Lymphocyte entry into lymphoid organs usually occurs via HEV located in the interfollicular regions [12]. HEV differ from normal blood vessels in their expression of vascular addressins. Thus, porcine HEV express higher levels of ICAM-1 (intercellular adhesion molecule) [85], whilst other blood vessels with high lymphocyte exit rates are VCAM-1 (vascular cell adhesion molecule) positive [14]. Interestingly, probably because of the inverted architecture of their lymph nodes, porcine lymphocytes also leave the nodes via HEV [53].

A number of molecules expressed on lymphocytes preferentially direct them to mucosal sites. The most important one seems to be α4β7-integrin, which interacts with MAdCAM-1 (mucosal cell adhesion molecule) on endothelial cells. Expression of α4β7-integrin has been demonstrated on memory T cells of sheep which migrate through intestinal lymph nodes and PP [41]. Likewise, high levels of α4β7-integrin were detected on lymphocytes in ovine

intestinal lymph, whereas expression in peripheral or pulmonary lymph was low [1, 42]. MAdCAM-1 was detected at high levels on HEV of mesenteric lymph nodes and PP as well as on postcapillary venules of the intestinal lamina propria, whilst tonsils and peripheral lymph nodes were only weakly positive [41, 66].

In contrast to MAdCAM-1, L-selectin preferentially directs the migration of lymphocytes into peripheral lymph nodes [15]. High L-selectin expression is seen on naïve T cells migrating to ovine peripheral nodes [1, 42], whereas lymphocytes isolated from bovine PP are L-selectin^{low} [15]. The L-selectin receptor, peripheral lymph node addressin (PNAd), is predominantly found on HEV of peripheral lymph nodes and tonsils [14, 66], but also in BALT of sheep with airway hyperresponsiveness, where PNAd is also involved in lymphocyte entry into the bronchoalveolar space [73]. Surprisingly, although lymphocytes in ovine pulmonary lymph express only low levels of both L-selectin and $\alpha 4\beta 7$ integrin [1], adhesion of ovine lymphocytes to pulmonary endothelia can be inhibited by treatment with anti L-selectin antibodies [39]. Hence, L-selectin levels are no direct indicator for lymphocyte migration rates. Although the percentage of L-selectin positive T cells is higher in milk than in bovine blood, expression levels in milk are actually lower, though this might also be due to L-selectin shedding [15, 31]. Similarly, although bovine $\gamma\delta$ T cells express higher levels of L-selectin than $\alpha\beta$ T cells, they rarely migrate to peripheral nodes [96].

Other molecules involved in the regulation of lymphocyte migration are CD44, LFA-1 (leukocyte functional antigen), $\beta 1$ and $\beta 2$ integrin [22, 101]. $\beta 2$ integrins (CD18) seem to be important for the activation step during entry into PP and other HEV-organs [14], and they are up-regulated during transendothelial migration in the mammary gland of sheep [65]. Treatment of lymphocytes with enzymes such as trypsin can inhibit their capacity to home to certain organs, although not all homing molecules are equally susceptible to enzymatic treatment [58]. Homing of IEL to their epithelial sites is mediated by $\alpha E\beta 7$ integrin binding to E-cadherin on epithelial cells [17]. Recently, a homophilic molecule expressed both on lymphocytes and epithelial cells, Ep-CAM, has been identified in the pig as a possible contributor to IEL retention within the epithelium [47]. In mice, the chemokine receptor CCR9 seems to partake in directing lymphocyte migration to the epithelium [94]. In general, chemokines and their respective receptors have been identified as key regulators of lymphocyte migration in mice and humans. Further research is necessary to determine to what extent these mechanisms apply to farm animal species.

To ensure optimal adaptation of the immune system, cell traffic is modulated during certain metabolic situations or after exposure to pathogens by altered expression of homing molecules or chemokine receptors. Additionally, lymphocyte migration can be modified by the nervous system. Stimulation of the greater splanchnic nerve in sheep results in increased lymphocyte traffic in mesenteric lymph [44]. Mucosal lymphocyte populations are differentially reactive to certain stimuli. For instance, dexamethasone treatment in pigs induces lymphocyte loss in mesenteric lymph nodes and the intestinal epithelial layer, whereas lamina propria and PP populations are not significantly influenced [86]. The hormonal changes during pregnancy, lactation or estrus also modify lymphocyte migration. Milk lymphocytes of cows up-regulate L-selectin, LPAM-1 and CD44 during the periparturient phase, whilst LFA-1 is down-regulated [31]. In pigs, prolactin can direct lymphocyte migration into the mammary gland [83], and progesterone treatment of sheep reduces lymphocyte numbers in the glandular epithelium and subepithelial stroma of the uterus [25]. Infection with pathogenic bacteria, viruses, or parasites evokes inflammatory responses, resulting in cytokine and chemokine secretion, which in turn influences leukocyte migration. As mentioned

above, the formation and maturation of functional BALT is especially dependent on infection [61]. Notably, mucosal inflammation is also associated with changes in adhesion molecule expression [80]. Alternatively, immunosuppressive mechanisms of pathogens can result in a reduction of lymphocyte traffic, e.g. intestinal lymphocyte migration can be downregulated during *Salmonella* infection in piglets [45].

6. THE ROLE OF LARGE ANIMAL MODELS IN BASIC IMMUNOLOGICAL RESEARCH

Lymphocyte migration has been studied in many species and important results have been obtained by using laboratory animals. The important role of large animals for the analysis of the mucosal immune system is that these species allow experimental procedures different from those possible in rodents. Therefore studies in large animals contribute to the knowledge about overall principles in lymphocyte migration and especially in the mucosal immune system. The typical routes of lymphocyte migration are comparable in all mammalian species, a peculiarity being the exit of lymphocytes from lymph nodes via the blood instead of efferent lymph that has been described in pigs.

Migration experiments in large animals use standard techniques for lymphocyte labelling, however, the use of syngeneic animals is limited. Sheep, cattle and pigs can be used for lymph vessel cannulation experiments, which enables the analysis of lymphocyte exit from an organ. Important results in large animal cell migration experiments are the description of naive and memory lymphocytes through lymphoid and non-lymphoid organs, the exit of lymphocytes from the PP and the follow-up of certain cell populations such as plasma cell precursors and γ/δ T cells. Although the number of markers useful for migration experiments is limited in large animal experiments, in recent years an increasing number of important markers and antibodies has been developed, and new molecular biological tools e.g. the use of tetramer technology will allow more detailed studies in large animals. "[…] many rich discoveries of biological diversity await fundamental research on larger species when it is more generally appreciated that the CBA mouse and the microtitre well cannot adequately represent the mammalian kingdom!" [12].

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