9. MIGRATION OF IMMUNE COMPETENT CELLS, HOMING AND EXTRAVASATION IN INFLAMMATION

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9.1. Introduction

Migratory behaviour of cells is one of the most essential cell physiological moieties of both unicellular and higher ranked organisms. The ability for active locomotion in cells representing primitive, early phases of cellular phylogeny has contributed to cells being able to distinguish between vital substances (e.g. food particles) and harmful, toxic agents in a sensible and efficient level. According to the theory of signal selection, receptors of molecular signals triggering cellular movement, as well as their intracellular signalling mechanisms have contributed to the fact that our cells are able to detect advantageous or harmful signals of their environment, and the big groups of the identical signalling mechanisms have also been separated. Molecules having special influence on the biochemical processes were selected from the simple food molecules, and big families of signalling molecules (e.g. hormones, cytokines, lectins) were formed, which could determine several normal and pathological reactions of the multicellular / human organisms.

Migration belongs, as a significant member, to the system of molecular networks described recently. This essential cell physiological reaction is a regulatory element of such activities as fertilization or angiogenesis, and migratory activity is also present in several basic processes of pathology and clinical sciences (inflammation, metastasis formation of tumors, atherosclerosis), as well as being a target reaction in some forms of therapy (selective drug delivery).

The objective of the following short summary is to give a schematic review on cell migration itself as a fundamental phenomenon of biology, and summarize the most important aspects of migration in immunology, pathology and clinics. At the end of the chapter, some methods are presented which are dedicated to measuring migratory responses of cells in laboratory practice.

9.2. Main types of cell migration

Migratory behaviour of the cells is classified in several ways in science. One of the most frequently used classification is the distinction made between kinesis and taxis. (i) ***Kinesis*** is a migratory response, when the direction of migration is not influenced by vectorial concentration of the inducer stimuli, it is a random response. (ii) In ***taxis***, the presence of the concentration gradient of the inducer is significant, the displacement of the cell is vectorial (*Figure 9.1.*).

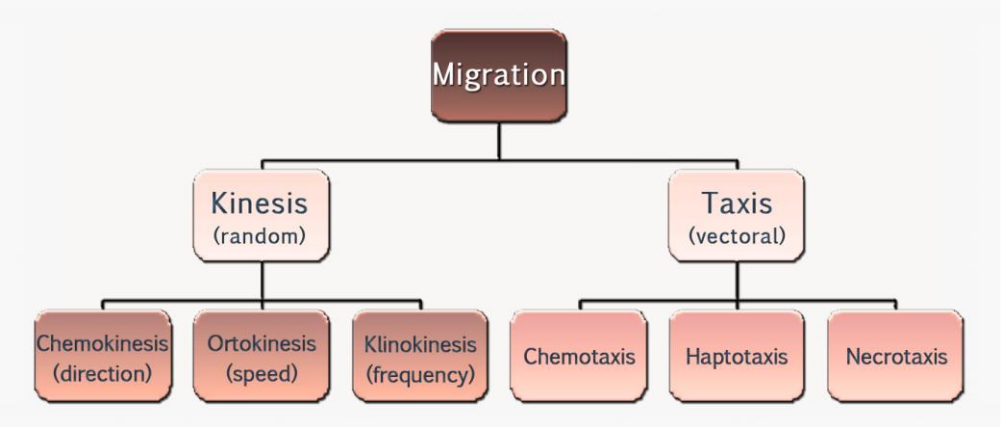

Figure 9.1. Main types of cell migration in prokaryotic and eukaryotic cells

Different types of 'kinesis' are distinguished according to whether (i) speed (orto~), (ii) frequency (klino~) or (iii) direction (chemo~) is the characteristic element of the complex migratory response.
In 'taxis' we distinguish (i) chemotaxis – the gradient of the inducer chemical is solved in the fluid phase; (ii) haptotaxis – the gradient of the inducer is associated with biological surfaces (e.g. endothels in blood vessels); (iii) necrotaxis – inducer materials are released from necrotic or apoptotic cells. (*Figure 9.2*).

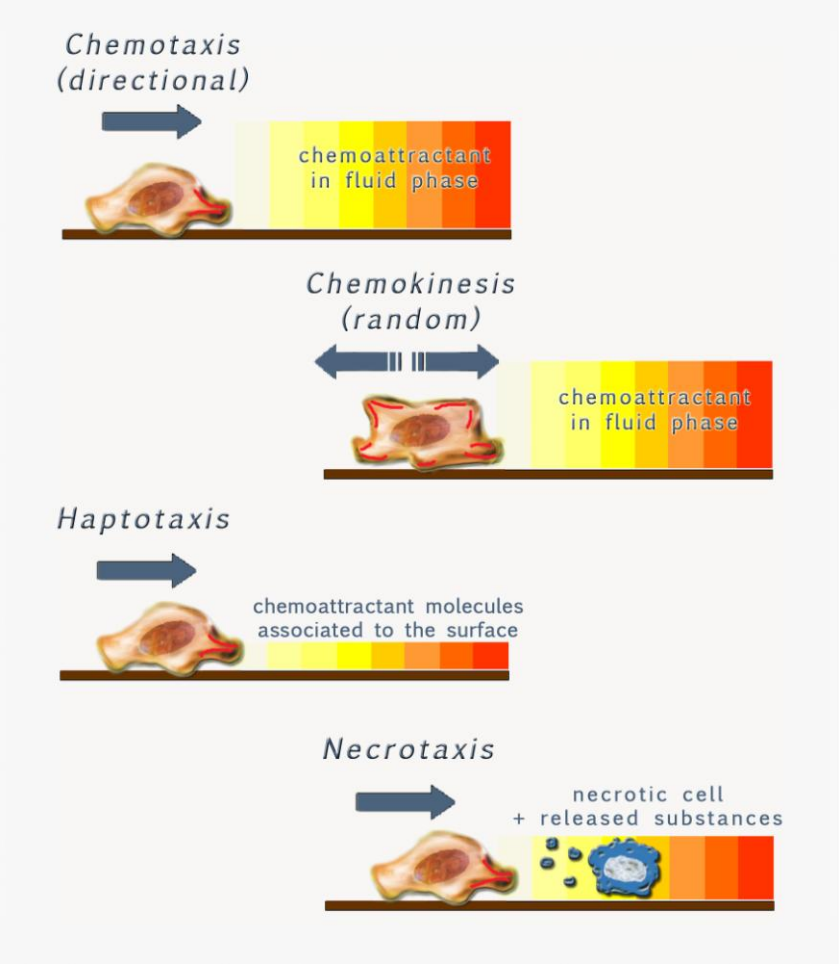

Figure 9.2 Migratory responses of eukaryotic cells induced by chemical substances

9.3. Molecules inducing chemotaxis

According to their effects on migratory cells, these substances are classified into two groups:

(i) chemoattractants – they elicit migratory responses of cells via increasing concentration gradient, they are considered as advantageous materials by the cells

(ii) chemorepellents – these molecules elicit migration via decreasing concentration gradient, they are regularly considered as harmful materials to the cell

The substances that trigger chemotaxis have a very wide range of characters. Very simple and small molecules (ions, amino acids) can be as strong chemoattractants / chemorepellent as the relatively big molecules (chemokines, synthetic drugs).

Characterization of chemotactic substances in biology and particularly in physiology, pathology and immunology has proved that there are professional chemoattractant molecules (e.g. formyl-Met-Leu-Phe, chemokines, complement 5a). However, there is a wide range of molecules the primary function of which is other than the induction of chemotaxis, and they still show chemoattractant / chemorepellent activity in some cells or special conditions (e.g. hormones).

The well-known representatives of the professional chemoattractants are chemokines. Four sub-classes of chemokines (C, CC, CXC and CX3C) are formed upon the presence of the peptide structure-determining elements: one or two disulphide bridges and the distance between the two central cysteins.

With respect to their function, the CX3C sub-class has a special character as its C-terminal domain is hydrophobic, which provides the possibility to be anchored into the surface membrane and to work as haptotactic ligands (*Figure 9.3*).

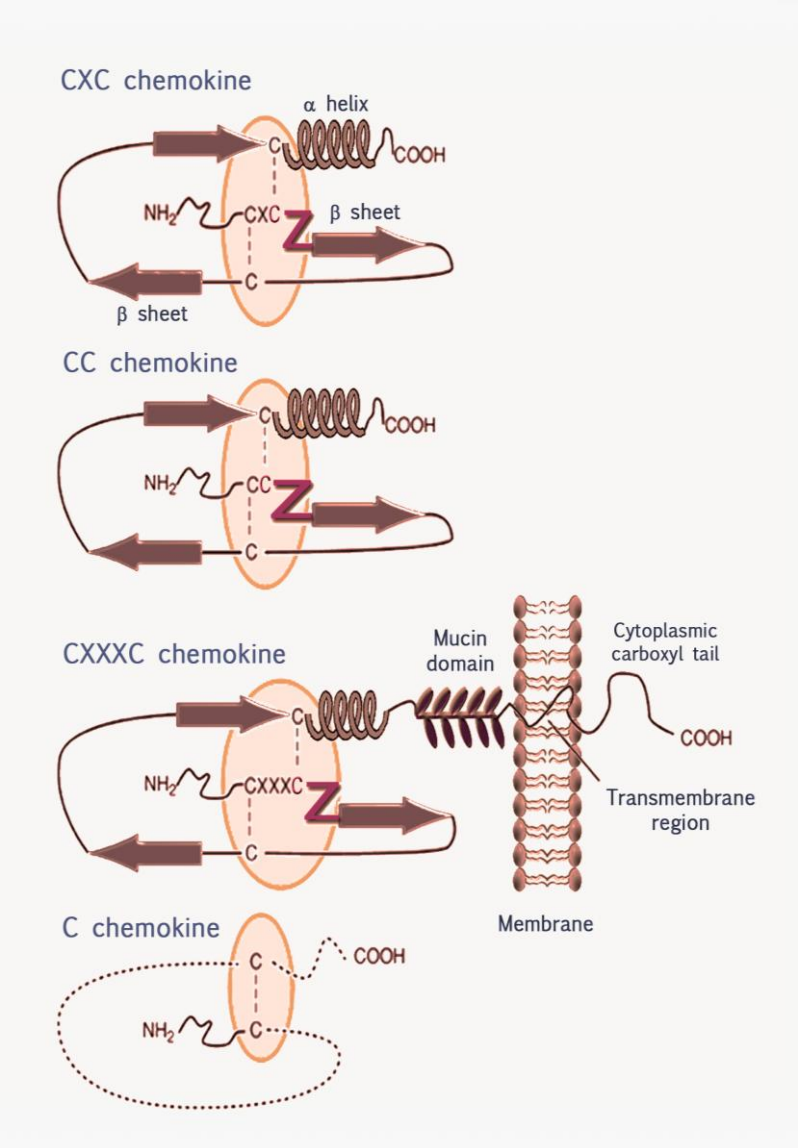

Figure 9.3 Classification of chemokines upon their molecular structure

9.4. Chemotaxis receptors

On the diverse levels of phylogeny, different ligand-membrane receptor interactions have been selected as the most effective molecular mechanisms triggering chemotaxis. In prokaryotes, the most effective chemotaxis receptors are transmembrane proteins (Asp-receptor, Tar-receptor), showing high affinity to relatively simple/small ligands (sugars, amino acids or their di- and trimers). Their four structurally distinct functional domains are: (i) ligand-binding domain extending to the extracellular space; (ii) „coiled coil" domain; (iii) a domain responsible for the activation of the receptor by binding of methyl groups and (iv) cytoplasmic domain responsible for starter phosphorylatoin of chemotaxis proteins of cytosol. Dimerization of chemotaxis receptors, which is frequently described at higher levels of phylogeny (e.g. chemokine receptors), is still present in bacteria and provides higher activity of signalling.

In surface membranes of eukaryotic model cells and especially in several human cells, we can distinguish a group of chemotaxis receptors which specifically bind to the short peptides containing formyl methionine (e.g. formyl-Met-Leu-Phe), released in bacterial infections. These receptors belong to the ***7TM receptor family*** and their ligand-binding is highly dependent upon the interaction between the ligand and the extracellular loops 1 and 2 of the receptor. Besides, the glycosylated extracellular sequence of the transmembrane domain 1 is still significant.

C5a peptide is bound by a similar 7TM receptor, however, in this case the extracellular peptide loop 3 and the domain 1 are responsible for binding. The intracellular signalling of the above mentioned two chemotaxis receptors is triggered by their intracellular C terminal part, which is associated with trimer G proteins. The most significant pathways of the intracellular chemotactic signalling network are PLC-PIP2-IP3-Ca^{2+}; Ras/Raf-MEK1/MEK2-ERK1/ERK2; MEKK-MEK3-p38-MAPK.

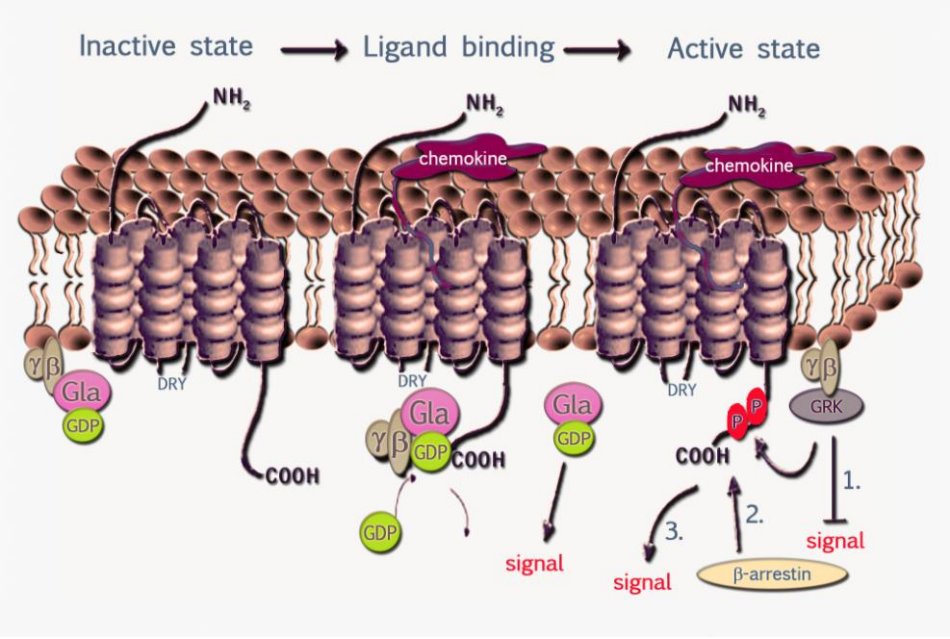

Figure 9.4 Signal transmission of chemokine receptors

Chemokine receptors are classified as 7TM receptors, too. The extracellular N terminal part of the receptor and the extracellular peptide loop 2 are significant in chemokine binding. In this case, the

intracellular signalling is triggered by a trimer G protein, however, in chemokine receptors a DRY (Asp-Arg-Tyr) peptide motif of intracellular loop 2 is also required. Activation of the receptor by GTP is helped by the intracellular C terminal part of the receptor – following association with beta-arrestin, a phosphorylation of the C terminal part generates the next signal. (*Figure 9.4*) glycosaminogylcans (GAGs) located on the extracellular membrane were also reported as significant members of chemokine-chemokine receptor interactions, as GAGs stabilize the ligand-receptor complex.

9.5. Motile cells and forms of their migration in human body

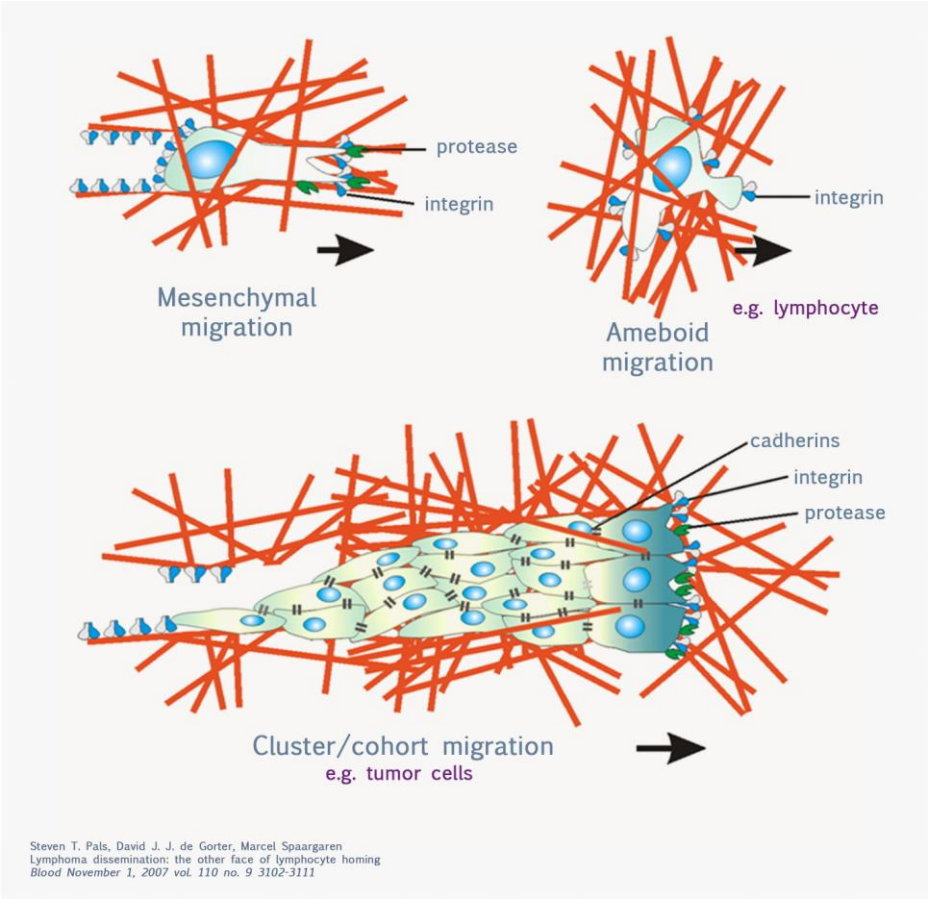

Figure 9.5 Main types of migration described in human

In higher levels of phylogeny, several cells are described as target cells of chemokines. In physiological and clinical aspects, the most important targets are: neutrophil granulocytes, monocytes, lymphocytes eosinophil granulocytes and the endothel of blood vessels.

The most significant migratory mechanisms of vertebrates are:

(i) Mesenchymal migration – The polarization of migrating cells is determined by the polarized expression of integrins and the release of proteases acting extracellularly.

(ii) Ameboid migration – A classical form of migration when the digestion of the extracellular scaffold has no significant role.

(iii) Cluster or cohort migration – A form of collective migration which was only described in the last decade. In this case, a group of cells anchored by cell junctional structures migrate together. This form of migration can be observed in morphogeny of embryonal tissues (endothel) and in some tumor growth, too (*Figure 9.5*).

9.6. Cellular motility and immune responses

In the immune system there are several cells possessing migratory behaviour, however, lymphocytes and dendritic cells (DC) are the best examples of the versatility of the process and the complexity of their cellular regulation. By this comparison we can follow the path of DC progenitors – immature DCs –, mature antigen presenting DCs which start from the bone marrow and arrive, via non-lymphoid tissues, to the immune organs (e.g. lymph nodes). *Figure 9.6* shows the above described process, which covers another significant migratory pathway – the circulation and migration of active T cells between the organs.

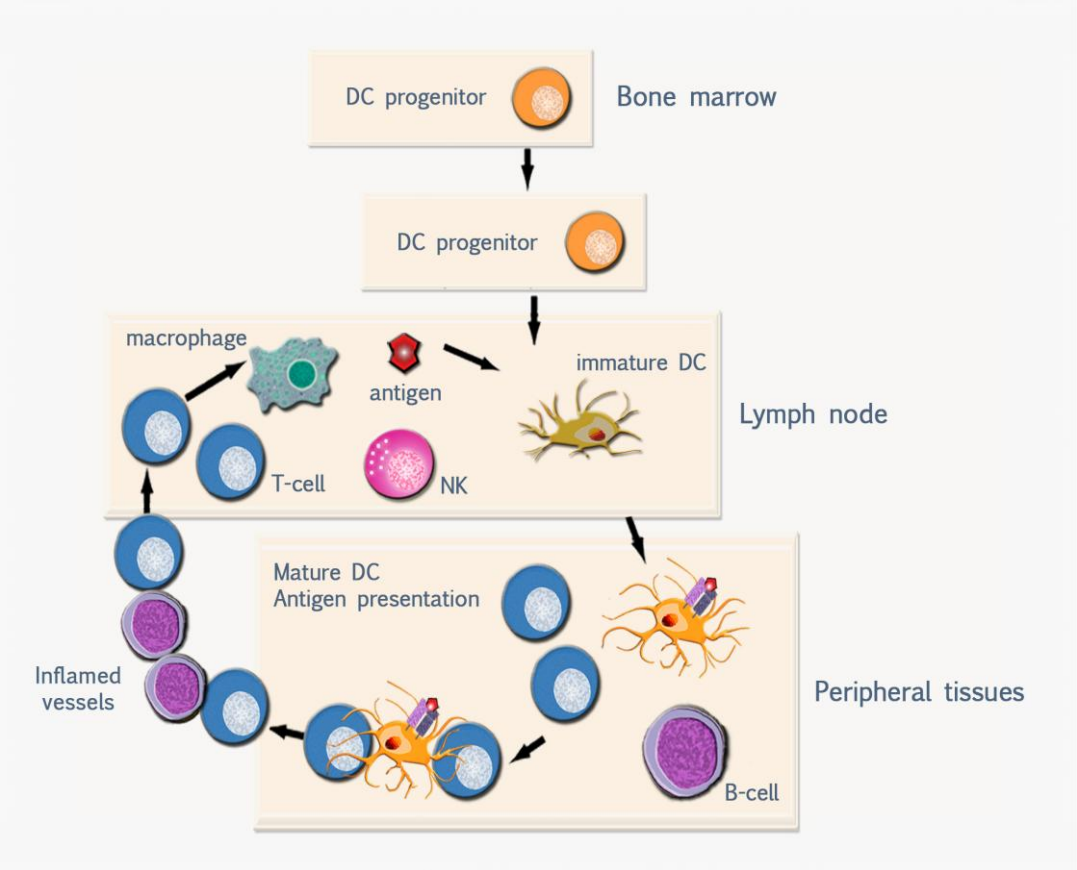

Figure 9.6 Main migratory pathways of dendritic cells and lyphocytes

9.6.1. Transendothelial migration of lyphocytes

The process outlined above is embodied in a much more subtle picture for each tissue spaces. In immunological and pathological processes, the most important surface where migration takes place is the endothel of blood vessels. Vectorial migrations (homing, inflammatory processes) of several cell

populations use these huge surfaces furnished with locally expressed membrane components (e.g. adhesion molecules) and inducer factors of fluid phase (e.g. chemokines or bacterial peptides).

In postcapillary venules, the phases of lymphocytic transmigration are accompanied by the following molecular interactions (*Figure 9.7*):

1. ***Rolling*** – interaction of selectins with sialomucins (or with integrin alpha4beta1, alpha4beta7)
2. ***Activation*** – chemokines are presented by the proteogylcanes of heparane sulphates (HSPG) on the surface of the endothel; lymphocytes are activated via chemokine receptors induced by chemokines of the endothel
3. ***Adhesion*** – activation results in lymphocytes with increased affinity and avidity integrins on their surface. These integrins are able to associate with their ligands (ICAM, VCAM etc.) expressed on the surface endothel. This interaction makes the adhesion firm.
4. ***Diapedesis*** – new molecular interactions develop with junctional adhesion molecules (JAM, PECAM-1=CD31), which results in the opening of interendothelial gaps and enables the transmigration of lymphocytes.

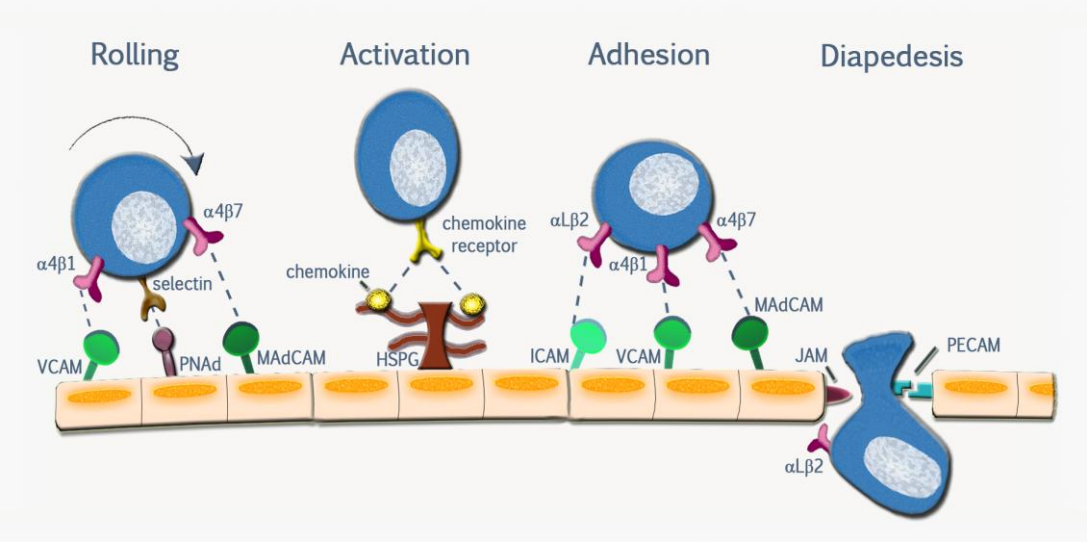

Figure 9.7 Main steps of transendothelial migration of lymphocytes

Migration of lymphocytes is controlled by adhesion molecules, chemokines and chemokine receptors. Homing of the ***naive T cells*** and their recirculation through secondary immune organs are possible pathways of migration as they express integrin alpha4beta7 in mucosa and L-selectin in lymph nodes. Migration of ***activated T cells*** to the site of inflammation is guided by several interactions of ligand-receptor pairs like: selectin-sialomucin, integrin alpha4beta1-VCAM-1, integrin alpha4beta1-CS-1 and CD44-hyaluronic acid. The homing signature of ***memory T cells*** is a specific protein profile of the surface membrane, composed of adhesion molecules and chemokine receptors, which help the selective and tissue-specific migration of these cells.

L selectin and alpha5beta7 integrin are co-expressed in naive B cells. These molecules enable naive B cells to reach mucosa and peripheral lymph nodes. Reactions running in the germinal centers of Peyer plaques result in the expression of alpha4beta7 integrin in ***memory B cells***. Part of these memory cells is differentiated to IgA producing plasma cells, while the majority of the B cells is derived

from the lymph nodes and differentiated to IgG producing plasma cells. The latter cells express CXCR4, alpha4beta1 integrin and LFA-1, which are essential for homing to the bone marrow where they will transform into 'long-lived plasma cells'.

9.6.2. Migratory pathways of dendritic cells

Following the efflux of dendritic cells (DC) from the bone marrow, they reach their target organs / tissues via circulatory system. Among target organs, thymus, lymph nodes, spleen and the skin are of special importance. The ***plasmocytoid DCs*** reach all four organs mentioned above, while thymus and spleen receive ***conventional DCs*** (cDC), and arrive in lymph nodes and skin as ***precursor DCs***. In the different organs, proper targeting of DCs is ensured by identical receptor-ligand interactions. In lymph nodes and thymus, the VLA4-VCAM1 and the PSLG-1 – CD62 (E/P) interactions have underlined significance, in the follicles of spleen the CXCR5-CXCL13, in the white pulp periarteriolar sheats the CCR7-CCL19/CCL21 interactions are the most characteristic ones. In the skin (and in inflammatory processes) a long list of molecular interactions is available, however, the most characteristic ones are PSLG-1 – CD62 (E/P), CXCR1-fractalcine, CCR6-CCL20 and CCR2-CCL2 relations.

In lymph nodes and skin, we can find good examples for molecular-level regulation of cell migration (*Figure 9.8*).

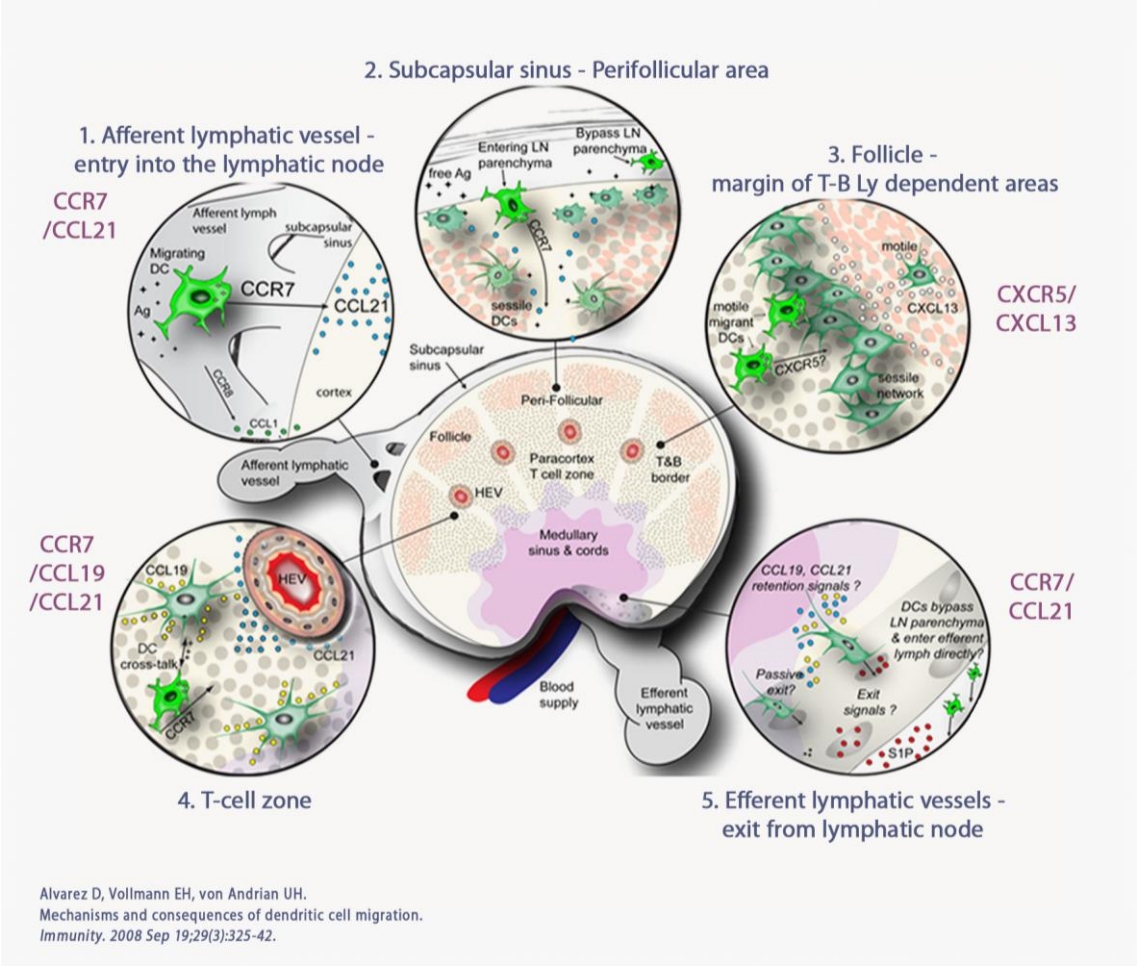

Figure 9.8 Migration of dendritic cell in the lymph node

(i) DCs arrive in lymph nodes via afferent lymphatic vessels (ii) to the space of subcapsular sinus, then their migration to the cortex is guided by the CCR7-CCL21 and CCR8-CCL1 chemokine receptor-chemokine interactions. (iii) When DCs reach the perifollicular space, their migration is directed by the CXCR5-CXCL13 chemokine receptor-chemokine interaction to the T- and B-cell-rich areas of the follicles. (iv) In the T-cell-rich zones the CCR7 positive DCs are accumulated around the high endothelial venules (HEV), the migration of which is triggered by the released CCL19 and CCL21 in the HEV area. (v) In the area of the efferent lymphatic vessels, sphingosine 1-phosphate works as an „exit signal", while the above mentioned CCL19 and CCL21 act as retention signals on DCs.

The skin provides another very good example of the migrating DCs of the immune system, however, in this case the DCs migrate in long distances: they patrol in the superficial epithelial layers of the skin and, as a consequence of antigen stimuli, they reach central parts of the immune system by active migration.

In physiological conditions, DCs of the epithelium are significant members of the immune system, being sensible to the molecules released from the cells attacked by microbes. These molecules (TNFa, IL-1b, PAMP) act as migratory signals on DCs. In the initial part of the above mentioned process E-cadherin linkages of DCs are unsealed and the cells become mobile. The next step is the breakdown of the basal membrane and its associated extracellular matrix elements by matrix metallo proteinases (MMP2, MMP9). At the same time, on the frontal part of the migrating DCs chemokine receptors are expressed (CXCR4, CCR7, CCR8), which are able to detect very low concentrations of the adequate chemokines (CXCL12, CCL21, CCL1), this way regulating the direction of migration. Under the skin, DCs arrive into the connective tissue where their target is the endothel of the afferent lymphatic vessels. Here the guiding signal relations are CCR7-CCL21, VLA-4 – VCAM-1, LFA-1-ICAM-1 and the above mentioned sphingosine 1-phosphate. Besides, the promiscuous chemokine receptor D6 of endothel also plays a significant role. The terminal step of the cascade is the internalisation of DCs into the lumen of lymphatic vessel where again CCL21 will promote their migration.

9.7. Techniques for measurement of cellular motility

As it has been presented in the chapters above, recording and measurement of cell migration allows us to analyse several changes in cell physiological conditions which are significant in immunology. By these measurement we can (i) isolate populations and sub-populations of cells; (ii) determine migratory activity of cell populations with reference ligands (e.g. IL8, fMLF); (iii) analyse chemotactic effects of body fluids or substances isolated from these fluids; (iv) carry out impact assessment of novel drug candidates or other significant substances with potential medical applications. Selection of the corresponding model-cells and the appropriate techniques requires great caution: although the list of the available techniques is wide, only a few techniques could provide the most appropriate responses to the well-formulated questions.

Figure 9.9 shows the two fundamentally different systems used to measure chemotaxis based on diverse migratory behaviour of cells (e.g. 2- or 3-dimensional forms of migration). In the ***reversible***

systems cells are located in a space where they are free to change the direction of migration up to the end of the assay, while in ***irreversible systems*** the choice of reversal migration is minimal as the cells and the attractant molecules are isolated by filters. In these systems, the stability of concentration gradients determines the optimal time of incubation which ranges from 10 min up to 24 hours. It is essential to know the optimal incubation time for each type of molecule to be tested: as soon as equalization of concentration gradient is completed, the system will no longer measure chemotaxis but chemokinetic responsiveness.

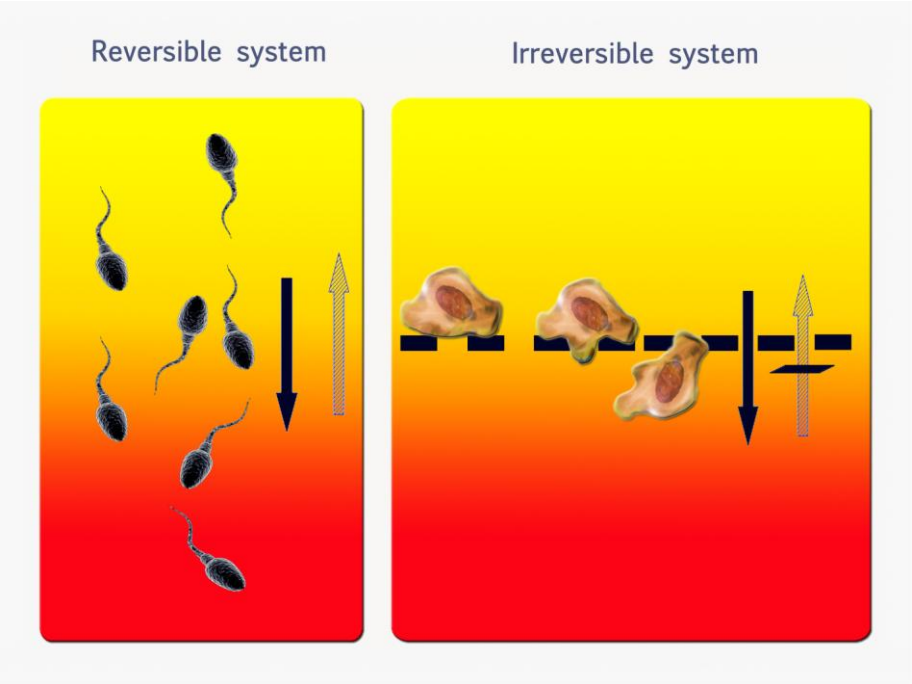

Figure 9.9 Main types of systems used to measure chemotaxis

There are several techniques dedicated to measuring the activity of migrating cells and the inducibility of chemotaxis. The information provided by the techniques and their efficacy is not always in proportion: the technique offering maximum information (Dunn chamber) allows measurement of relatively few cells, while receptor binding assays provide only a single data, although the number of measured cells is over 10^6.

As the facts above suggest, multi parametric assays are the most effective, applying different techniques to evaluate substances or chemotactic responsiveness of model cells. In the following section you can find examples of the most frequently used technical approaches of the problem, and we will draw the reader's attention to the available literature in the field.

9.7.1. Reversible systems

This group of techniques can measure migratory responses of cells moving by cilia, flagella or pseudopodia of ameboid movement.

According to the references, the longest stable concentration gradients (about 24 hours) are provided by ***Dunn chamber*** (*Figure 9.10.*). The figure shows the cross-section of the chamber which is composed of two ring-shaped containers. The migration of cells takes place on the connecting bridge.

The concentration gradient develops on the tiny fluid layer which connects the two containers and the number of positive responder cells is also counted in this part of the chamber.

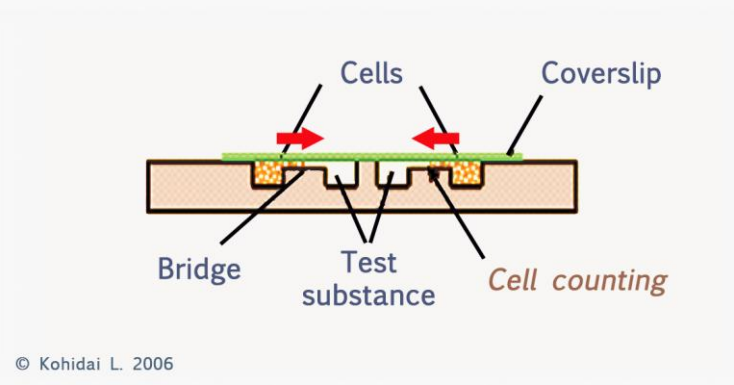

Figure 9.10 Structure of Dunn chamber

The ***agar-plate technique*** is one of the oldest techniques to detect displacement of cells on surfaces (*Figure 9.11*). The presented method is only a representative one, there are several others dealing with channels and wells in the agar layer. The basic theory of the present one can be deducted from other similar techniques of immunology, where interactions of antibody and antigen take place in the agar. In the present case, the cells to be tested are placed into a central well, while substance to be tested and a control material are loaded on the two sides. The cells migrating mainly under the agar layer and the changing symmetry of a cloud-like pattern show whether the substance is working as an attractant or a repellent one. By measuring distances on the cloud-like pattern (see d_A and d_C), we can also turn the assay into a quantitative one.

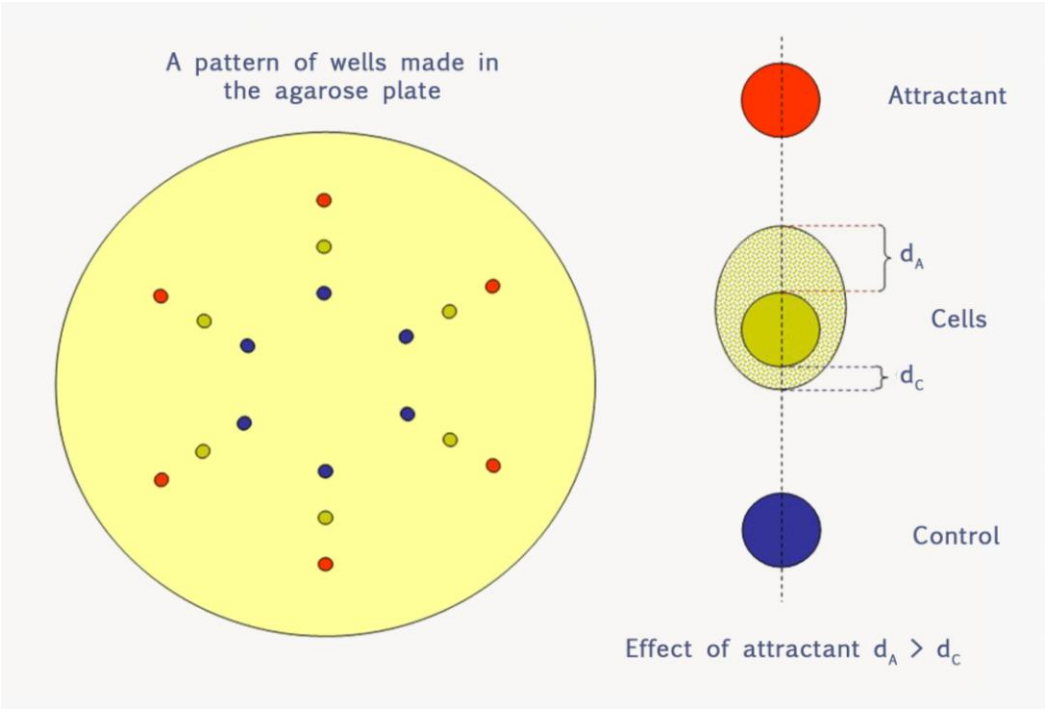

Figure 9.11 Three-hole agar plate technique for measurement of chemotaxis

Capillary techniques are frequently used to evaluate chemotaxis of cells migrating with cilia or flagellum in the free and undivided fluid phase. In general, these assays use vertically arranged two-chamber systems, where the cells to be tested are placed into the lower chamber and the test substance is applied in the upper chamber which is a capillary. The attractant or repellent effect is

determined by counting cells in the capillary and relating this data to a reference value. Accuracy of the method can be significantly increased when micropipettes working with microlitre precision fluid volumes are used as capillary part of the system.

Application of serial chemotaxis assays is increasing in the everyday practice as their accuracy ensures high efficacy of the measurements. In recent years, two dedicated chemotaxis chambers have appeared on the market (ibidi® GmbH, Germany), which can measure chemotactic responses of cells migrating of surfaces (***μ-slide***) and in semi-fluid conditions (μ-slide 3D) (*Figure 9.12*). The essence of the chambers is a system of communicating channel and two chambers, where the continuous exchange of gaseous materials is provided by a special plastic coverslip. The cells are loaded into the central channel while the two chambers are filled with the test and the reference substances. After 12-24 hours of incubation, on the two sides of the channel we can measure significant differences in cell numbers. Application of video-microscopy and cell-tracking are also viable ways of evaluating chemotactic responses of cells.

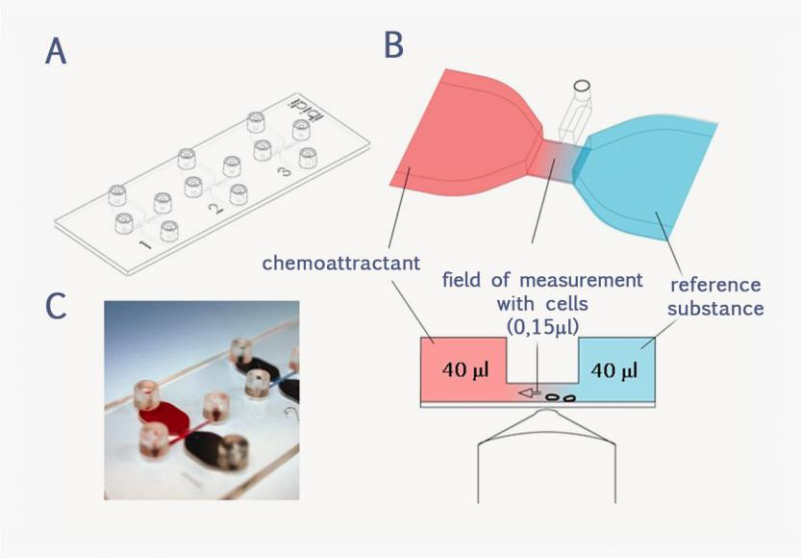

Figure 9.12 Structure of ibidi® chemotaxis chamber (A) and the principle of its operation (B, C)

9.7.2. Irreversible systems

Probably the ***Boyden-chamber*** described by Stephen Boyden in 1962 is the most frequently applied technique to measure chemotaxis (*Figure 9.13*). It is also a two-chamber assay, where the chambers are isolated by a filter with a proper pore size. The cells are placed into the upper chamber while the test substance is in the lower one. Cells cross the filter by active migration as they detect the concentration gradients developed in the pores of the filter. The Boyden-chamber is available not only as a single one but also in multi-96-well (high throughput system – HTS) for, making it possible to test samples in the required high number of parallels. For evaluation of the migratory responses, oculometric counting, detection of cell-specific enzymes (e.g. mt-dehydrogenase by MTT assay) or measurement of ATP levels are the most frequently applied methods. The disadvantage of the Boyden-chamber is that the evalution represents only a single measuring point, therefore reliable results could be obtained only when we evaluate several parallels.

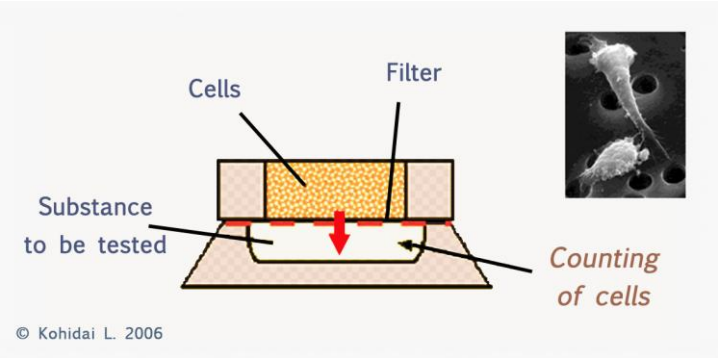

Figure 9.13 Structure of the Boyden-chamber and the principle of its operation. The right panel shows cells migrating through the pores of filter

Disadvantages of the Boyden-chamber discussed above are expected to be eliminated by the ***transwell assay*** in 6-12-14-96 well capacity designs. Its principle is similar to the Boyden-chamber: it also has two chambers isolated by a filter. Cell number in the lower chamber or in the filter itself shows the positive responsiveness of cells, evaluation of the filter is similar to a histological section. Disadvantage of this technique is that it is very hard to equalize the fluid levels in the two chambers. Even a barely visible difference might result in undesired transient fluid flows that can contaminate the cell-rich compartment with high concentrations of test substance or break the development of concentration gradient.

The most recent technique in this field, the ***Chemo Tx assay*** (NeuroProbe, USA) combines the advantageous moieties of the Boyden-chamber and the transwell systems. The hermetically sealed space eliminates any undesired flows. The reusable (multiwell) variant of the chamber (*Figure 9.14. Left panel*) is the conventional type, while a disposable chamber is also available since 2008 (*Figure 9.14. Right panel*). Evaluation of the chambers is similar to the techniques described at the Boyden-chamber.

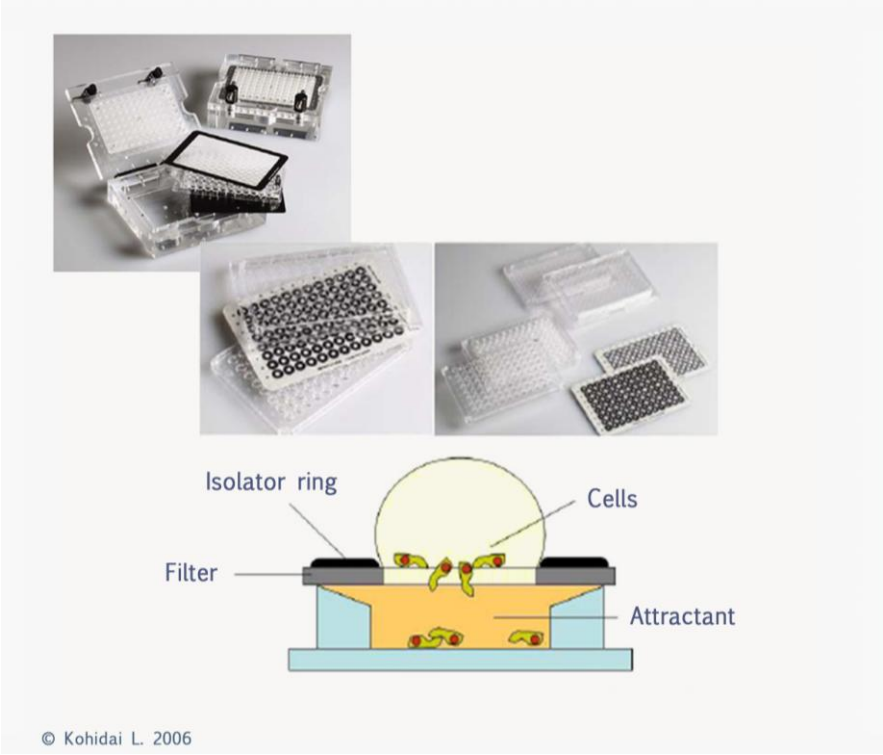

Figure 9.14 Multiwell chemotaxis assay and Chemo Tx technique

9.7.3. In vivo techniques

Matrigel is a concentrate of extracellular matrix (ECM), which is frequently used as an artificial tissue and network for migratory cells of whole animal experiments. This jelly-like material is used by the so-called disc assays, when a plastic ring sealed by filters and filled by Matrigel is implanted into the animal's body. Migration of cells into the disc usually takes several days, after which the cell number or ratio of different cell populations is determined in the removed disc. *Table 9.1* shows that, in addition to the mixture of ECM-composing Matrigel, there are several growth factors, too. These components are present in significant concentrations to elicit biological effects, however, in some experimental cases this kind of content can be explicitly detrimental. Recognition of these circumstances has led to the introduction of "growth factor reduced" GFR-Matrigels on the market. As data suggest, the product is not free of growth factors even in this case, but it is understandable as these factors are responsible for the enhanced chemoattractant character of Matrigel, and work as humoral angiogenic elements on cells migrated into the gel.

Growth factor	**Concentration in Matrigel**	**Concentration in GFR Matrigel**
EGF	0.5-1.3 ng/ml	< 0.5 ng/ml
bFGF	< 0.1-0.2 pg/ml	n.d.
NGF	< 0.2 ng/ml	< 0.2 ng/ml
PDGF	5-48 pg/ml	< 5 pg/ml
IGF-1	11-24 ng/ml	5 ng/ml
TGF-b	1.7-4.7 ng/ml	1.7 ng/ml
GFR – growth factor reduced; n.d. – non detectable		

Table 9.1 Growth factor content of Matrigel products

Another ingenious in vivo technique is when Sephadex beads are introduced into the peritoneal cavity of the animal. The beads induce the plates of peritoneum, and migrating cells are accumulated in the peritoneal fluid. As the material of beads is a dextran gel, it has high surface binding capacity. In chemotaxis assays, these binding properties are used to trigger the migration of special subgroups of cells (eg. lymphocytes) by ligands associated to the surface of the beads. The incubation time is 6-48h, for evaluation the drained peritoneal fluid is used.

Wound-healing assays are also discussed in this part, however, they are regularly considered as an independent form of chemotaxis assays. Generally confluent epithelial layers are used in these probes, where the speed of "healing" (two wound margins getting closer and closer to each other) is recorded after "wounding". The original way of "wounding" applied pipette tips to scratch lines. A more recent and reliable method can prepare wounds by a single well-controlled displacement of a 24-48-96 needle platform. In the last 5 years – similarly to the two-chamber assays –, high throughput techniques have been getting into the focus. Short impulses of an appropriate current are also able to form "wounds" in cell layers. For evaluation of these assays, impedance-based techniques are applied (see below).

9.7.4. Recent techniques

In the introduction of the above mentioned ***impedance based measurements***, the equipment (ECIS) developed by the Nobel laureate Ivar Giaver was a pioneer one. This technique is dedicated to measuring cell adhesion as well as the migratory behaviour of cells. The principle of the method is that the cells stuck onto the surface of measuring electrodes work as insulators in AC, and result in increased ohmic resistance (R) and impedance (Z) as well. Based on this phenomenon, the adhesive character of cells or its inducibility / inhibition is well detectable. The method can also register the fine fluctuation of the impedance, which is a good mirror of micromotion of cells. One of the main values of this technique is that measurements run in real-time and we can follow changes for several days in a sample (*Figure 9.15*).

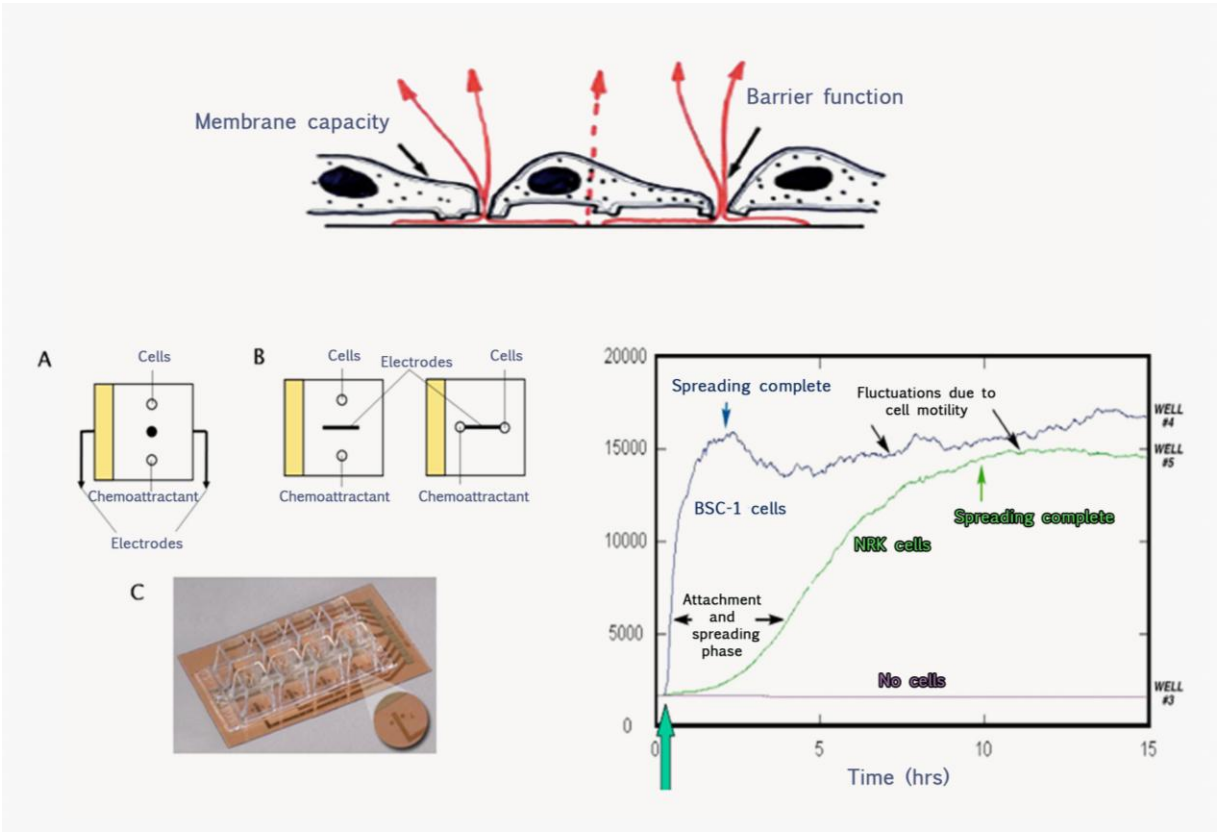

Figure 9.15 Theoretical principles of ECIS. The method helps to evaluate the level of coverage of electrode (cell number) and the cell-cell connections (barrier function) (top panel); measurements are carried out on small surface electrodes in 8-16-96 well platforms (left bottom panel); by this technique it is easy to distinguish the differences in adhesion dynamics, as well as the identical motions of the cells (right bottom panel).

As further development of the above technique, different HTS systems have been designed (e.g. xCELLigence – Roche), the principle of which is derived from the Boyden-chamber. In these systems, the isolator filters of the two chambers are furnished with electrodes, which makes it possible to register the transmigration of cells by the generated electric signals. The measured impedance values are in good correlation with the number of cells crossing the membrane.

Application of point-shaped electrodes has enabled the technique to remove cells above the electrode (see wound-healing assays). As it is easy to follow and register the dynamics of cell migration into the cell-free surface, this way of evaluation represents the most objective tool for the measurement of wound-healing assays.

One basic requirement of the objective chemotaxis assays is the development of consistent gradients in the fluid phase. Tools of the novel technique, microfluidics could help to achieve these standards. In the present case, ***polydimethylsiloxane (PDMS)*** is used to form channel systems which can dilute the test substance with high accuracy. PDMS technique is the fundamental element of building "lab-on-chip" systems, too, which enables the implementation of whole laboratory work-flows in a slide dimension analyzer unit. The assay shown on *Figure 9.16* is a PDMS based system (see lower panel with steps of fabrication), composed of two horizontally arranged chambers and a connective channel with a pressure sensible late on the top of it. Having loaded the two chambers, an adjustable negative pressure is applied via central channel. By changing the pressure we can adjust the mixing rate of the attractant and the slope of the concentration gradient. After the development of the gradient, the cells are also loaded and their concentration dependent migration can be examined.

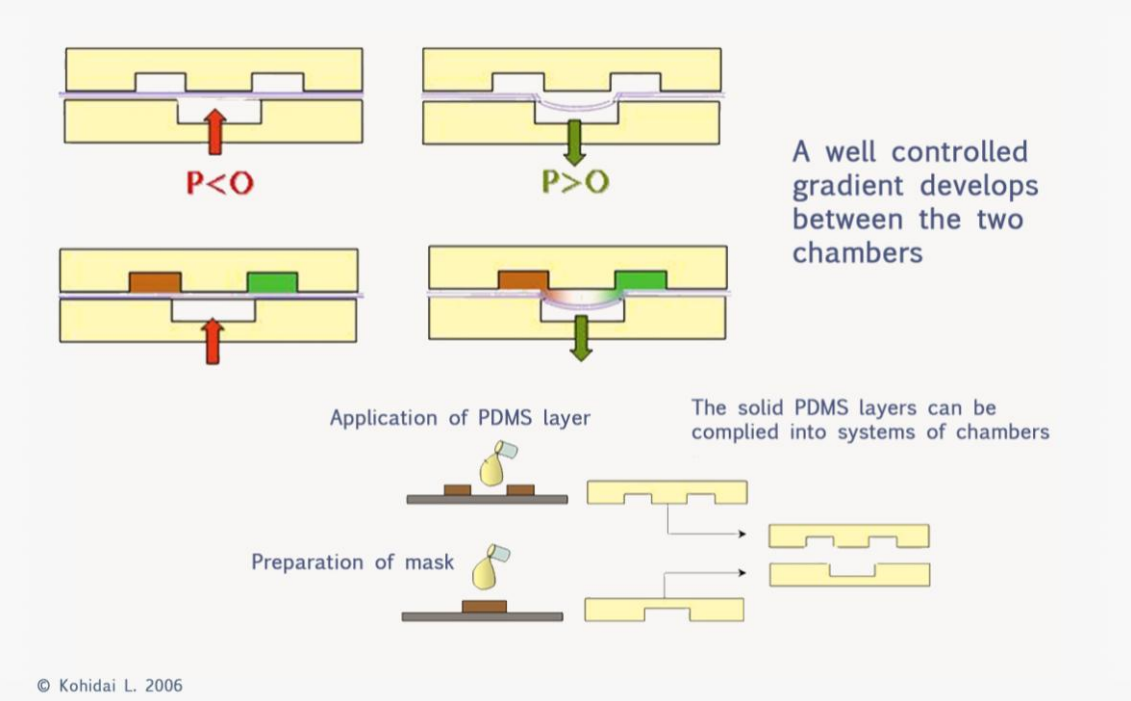

Figure 9.16 Chemotaxis chamber developed by PDMS technique.
The main steps of fabrication of the profiles using masks and PDMS are shown on the bottom panel.

Intravital microscopy (IVM) is also well adaptable to studying migrating cells. The technique is suitable for the investigation of whole animals or large organs in vivo, without the application of fixatives or anticoagulants. The heart of the technique is the combination of an extremely high shutter speed camera with a computer which provides the possibility to register especially vascular events like adhesion of cells to the vascular endothel and transmigration.

At the end of this chapter you can find one of the most sophisticated and sensible techniques for studying the displacement of cells – traction force microscopy (TFM) (*Figure 9.17*). Theoretical basics of the technique are derived from recording forces generated between the migrating cell and the surface. This means that focal contacts of the cell are the points where the forces are exerted onto the layer below the cell. These interactions can be measured by a multitude of microneedles, the direction of their bending is identical with the negative vector of migration, while the level of bending is identical with the force generated. The dimension of the forces measured by this technique is on nN scale in the case of human neutrophyl granulocytes (PMN).

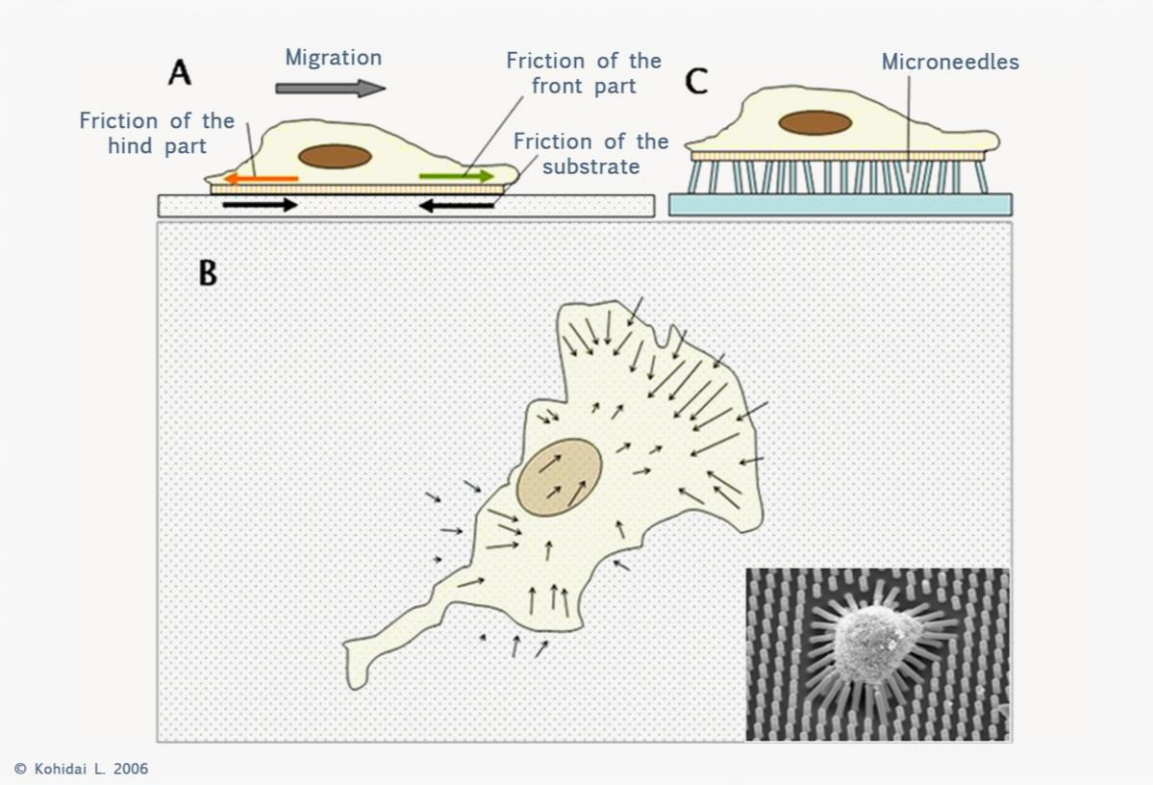

Figure 9.17 Vectors of forces registered by traction force microscopy (TFM) in migrating cell (A); distribution of these forces on different parts of the migrating cell (B); interaction of migratory cell and microneedles (C and right bottom panel).

9.7.5. Technique associated with migration assays

The investigation of migrating cells obviously does not only mean recording and evaluation of the migratory response, but also molecular level detection of receptors and ligands, as well as genome and transcriptome level analysis of the responsible genes. In the case of RNA expression, in the ***RNase protection assay (RPA)*** technique first the RNA is hybridized to its labelled antisense. Then an RNase is applied to eliminate the single stranded RNAs from the sample. This way it is easy to isolate the double stranded and labelled RNA molecules from the other components. Dealing with RPA, several kits are available including kits for chemokine families and chemokine receptors, too.

9.8. Questions

1) Which kinds of migratory responses are present in the human body?
2) Which are the most significant groups of chemoattractants in the human body?
3) What are the most important structural and functional differences between the chemokine sub-classes?
4) What kind of signalling mechanism(s) are characteristic to the chemokine receptors?
5) Please give the definition of 'Collective migration'.
6) What are the most important receptor-ligand interactions in regulating DC migration in lymph nodes?
7) Which are the chemotaxis assays where evaluation is possible even on HTS level?
8) Is the human body furnished with chemotaxis receptors specific to bacterial peptides?
9) Which are the theoretical principles of ECIS technique?
10) Please give example(s) for the 'lab-on-chip' based measurement of migration.

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