Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity

Summary: This review summarises experimental evidence to illustrate that induction of immune reactivity depends upon antigen reaching and being available in lymphoid organs in a dose- and time-dependent manner. If antigen reaches lymph organs in a localised staggered manner and with a concentration gradient, a response is induced in the draining lymph node. Antigen-presenting cells are of critical importance to transport antigen from the periphery to local organised lymphoid tissue. If antigen is all over the lymphoid system, then it deletes all specific cells in the thymus or induces them within a few days; because of their limited life-span they then die off, leaving the repertoire depleted of this specificity. If antigen does not reach lymphoid organs it is ignored by immune cells. Once a response is induced, activated but not resting T cells will reach antigen outside lymphoid organs, whereas activated B cells differentiate into plasma cells in an inducing environment, mostly in lymphoid tissue including bone marrow, but also in chronic lymphoid-like infiltrations in peripheral organs. In immunopathology (when the infectious agent is known) or in autoimmunity (when the triggering infectious agent is not known or not recognised) lymphoid tissue may become organised close to the antigen (e.g. in organ-specific autoimmune diseases) and may thereby maintain an autantigen-driven disease-causing immune response for a long time. The notion that naive T cells get induced or silenced in the periphery may be questioned because induction can only occur in lymphoid organs providing anatomical structures where critical cell-cell interactions are properly guided and where, therefore, cells are likely to meet sufficiently frequently and in a critical milieu. Since overall immune reactivity critically depends upon the localisation of antigens in a dose- and time-dependent manner, it seems more likely – but this remains to be shown – that activated T cells may get exhausted in non-lymphoid peripheral tissues, whereas they are usually maintained in lymphoid organs. The critical role of antigen in regulating immune responses also has relevance for our understanding of immunological defence against epithelial and mesenchymal tumours, against many infectious diseases and for understanding autoimmunity and immunological memory. Collectively the data indicate that antigen, dependent upon localisation, dose and time, seems to be the simplest regulator of immune responses.

General parameters of the immune system

The major function of the immune system is to defend vertebrates against infections (1–6). To achieve this goal, the two main arms of the immune system, cellular and humoral immunity, very efficiently cooperate and functionally supplement each other. The main targets of T cells are MHC-bound peptides derived from intracellular antigens and infectious agents, while
Fig. 1. Induction of immune responses depends on antigen localisation in a dose- and time-dependent fashion.
A) Indifference/ignorance: If antigen stays in peripheral solid organs (e.g. self-antigens of the β-islet cells (βI) of the pancreas or MBP in the brain) and/or in non-migrating cells (e.g. rabies virus (VR) in neuronal axons or Papilloma virus (VP) in keratinocytes), these antigens or infections are immunologically ignored. Similarly, no immune response is induced against epithelial and mesenchymal tumours (sarcomas (Sa) and carcinomas (Ca)) that do not reach organised lymphoid tissue.

B) Induction of immunity: Immune responses are induced if antigen reaches secondary lymphoid organs. This usually happens in a staggered fashion when a virus infects the skin of the big toe (V1), replicates first locally (1–3 days), then spreads in migrating APC to the local lymph node (V2), where it further replicates and induces T cells and B cells. Hematogenic spread then may generalise infection to many organs (V3).

C) Exhaustion: In the situation where non-cytopathic virus or endogenous virus is transmitted during pregnancy to offspring, T cells are deleted. T cells may also be deleted when a virus spreads within a few days throughout the host and induces all specific T cells to become effector T cells, which die off within 1–3 days. Such overwhelming infection thereby exhausts the T-cell response.

D) Peripheral persistence: A potent and efficient immune response against a virus usually eliminates most viruses. Viruses may, however, persist in cells of peripheral solid organs (V4), such as kidney tubular cells (cytomegalovirus) in parotis epithelial cells or in neuronal cells (Herpes simplex), and are not eliminated definitively, because antibodies have no access and T-cell activation is insufficient to reach these sites. From these sequestered sources viruses may spread periodically (V4) to lymphoid organs and restimulate T and B cells to control interval viruses or reinfection from the outside (infection immunity).

antibodies target primarily extracellular agents. However, immune defence is not completely efficient against all pathogens; several pathogens may escape immune surveillance for various reasons. Also, the immune response against a given pathogen may not completely succeed in its elimination from the host or in the prevention of damage. As for most biological equilibria, interactions between the immune system and pathogens are therefore successful in the order of 90–98%, leaving only a few very unfavourable situations.

If we consider virus infections, the various biological parameters, in particular the degree of cytopathogenicity of different viruses, demand distinct strategies of the immune system in order to achieve successful immunological protection. Four general types of host–virus relationship may be defined: i) cytopathic viruses which destroy vital host cells rapidly; to assure the host’s survival, rapid elimination by the immune system and other resistance mechanisms are a prerequisite (e.g. polio viruses); ii) some cytopathic viruses can persist after the acute phase of the infection and hide in cells by various mechanisms (e.g. Herpes Simplex Virus I in neurons) and may periodically replicate and become cytopathic; these viruses harm the host only to a rather limited extent and have established a complicated but mutually acceptable relationship; iii) viruses that are either non- or poorly cytopathic can infect cells without endangering the health of the host and may therefore induce a chronic carrier state; these viruses productively infect cells (e.g. lymphocytic choriomeningitis virus (LCMV) in mice) and some may also be integrated into the genome of host cells (e.g. Hepatitis B virus (HBV) in humans); iv) endogenous viruses, which are non-pathological and are immunologically part of the host self (e.g. MMTV or endogenous viruses in the mouse).

The proposal
Our geographical view of the immune response proposes that antigen alone regulates immune responses, dependent upon antigen localisation, antigen dose and time (Figs. 1 and 2).

Some necessary definitions
For the derivation, justification and discussion of our geographical view of the immune response some definitions need clarification.
Antigen Localisation

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- slow → Ignorance 2A
- rapid → Response 2B
- overwhelming → Exhaustion 2C
- persistent → Infection 2D
  
**Antigen Kinetics**

**Fig. 2. Schematic depiction (2A, 2B, 2C, 2D) of the antigen localisation, dose and kinetics of the four different situations shown in Fig. 1A, 1B, 1C and 1D, respectively.**

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**Immunogeographical parameters of the host**

Organised lymphoid tissue. Lymphocytes, macrophages and APC may perform their effector functions as single cells, but in order to be activated they need to meet and collaborate in organised lymphoid tissue (7–12). The term organised defines structures such as follicles, marginal zone, germinal centre, periarteriolar sheath, red pulp and channelled pathways that direct lymphohemopoietic interactions (7, 9, 11). These anatomical structures determine the localisation of antigen, cytokines, interleukins and bystander contacts via accessory molecules, i.e. they provide the essential parts of the milieu necessary for lymphoid cell interactions. These cell interactions are ordered anatomically and in a timely sequence around structures that concentrate antigens. Specific encounters of cells with antigen as such are thereby controllable and strongly enhanced. Since some APC are specialised in retaining immunogenic antigen, the availability of antigen to stimulate T and B cells is often considerably prolonged (11, 13). Thus, secondary lymphoid organs present antigen optimally and enhance the chances of specific antigen encounter and of specific cell interactions. (As will be discussed in detail, these requirements render chance encounters of antigen by lymphocytes and activation anywhere else extremely inefficient and of no biological relevance; this is an important safeguard against induction of autoimmunity.)

The lymph node and spleen fulfill the stated requirements best, while primary lymphoid organs, including the thymus and bone marrow, do not usually participate in the induction of T or B cells. The lymph nodes function as recipients of antigen that is transported by dendritic cells or macrophages via afferent lymph from peripheral solid organs (14). The spleen functions as a filter of the blood for soluble or particulate antigens that are not cell-associated or for cell-associated antigens that are predominantly present in the blood (15, 16).

Peripheral solid organs. These are organs that are themselves not organised lymphoid tissues. When extended to a cellular level this includes cells that do not as a rule migrate to organised lymphoid tissues (e.g. fibroblasts of the big toe, parenchymal cells of the ovary, the lung, kidney or thyroid epithelial cells or β-islet cells of the pancreas). One cell population requires special consideration, namely antigen-presenting cells (APC), which are not part of the peripheral solid organ in the definition used here. They migrate from peripheral solid tissue to organised lymphoid tissue and transport self-antigens and foreign antigens, including infectious agents. On the other hand, fibroblasts in organised lymphoid tissues (including lymph node and thymus) are part of the organised lymphoid tissue as defined here.

Lymphocyte circulation and migration pathways. Within the defined geography cells move according to their activation status (8). Naïve lymphocytes circulate in blood, lymph nodes and spleen, but do not emigrate and do not migrate through peripheral solid tissues. In contrast, activated T cells are able to emigrate into peripheral solid tissue, particularly to inflammatory lesions. Their fate in the peripheral tissue seems uncertain. It is unclear whether they always die or whether they migrate back into the afferent lymph or into the blood circulation.

Activated B cells seem to home to the red pulp of the spleen or the bone marrow (15, 17). Antigen-presenting cells (APC) are cells that migrate from peripheral sites to organised lymphoid tissues to present transported antigens to T and B cells (14).

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**The geography of antigen entering the host**

**Localised antigen.** This term describes the fact that normally infections start at one location in the periphery, spread from there to the local lymph node within 1–3 days, and from there, after a delay of a few days, generally throughout the organism (5, 18). The use of an adjuvant together with an antigen imitates this general kinetics; this becomes most obvious with adjuvant containing bacterial components inducing a granulomatous inflammation that retains antigen. The time kinetics of this staggered infection cascade and spread of infection contrasts with an early hematogenic spread of an infection, which is a relatively rare event under physiological conditions (e.g. Arthropod-born viruses reaching the blood via insect bites or contaminated blood transfusions).
Antigen localisation, dose and time kinetics. These terms will be used to indicate that they form a three-dimensional integral that determines the immunological response. i) Following the consideration outlined above, antigen may be localised in organised lymphoid tissue or in the peripheral solid organs. However, it may move from periphery to lymphoid organs on APC. Antigen localisation – particularly in infections – should therefore be regarded as a dynamic process, which includes a quantitative and temporal aspect. ii) The temporal aspect of antigen distribution implies that antigen has to persist for a critical time in lymphoid organs to induce T cells. In addition, it is important how fast antigens will distribute through the host. The optimal distribution kinetics is achieved in initially localised peripheral infections, as outlined above. After low-dose intravenous infections this overall kinetics is still preserved to a large extent and is similar to a peripheral infection. After high-dose intravenous infections, however, antigen is everywhere within two or three days and causes exhaustion of the T-cell response (see below) (19). iii) This obviously depends on the antigen dose, which is a third co-ordinate in the dynamic process of antigen distribution. Antigen dose not only reflects the actual immunising dose, but in the case of pathogens also depends upon the speed of replication.

Thus, antigen localisation is a complex dynamic time- and dose-dependent process, which is best described by a three-dimensional integral of the three parameters. In the next section we will point out the importance of these three parameters for the induction of an immune response.

**Antigen as immune regulator**

The question of how immune responses against pathogens are induced is intimately linked to the questions of how immune responses against self-structures are avoided. Two general concepts of immunity have been proposed to explain these fundamental issues:

1) Negative regulation. There is a symmetrical regulation of the immune system by stimulation (i.e. help) on one side and suppression on the other side. This intuitive view postulates regulation as an a priori requirement; it has been most elegantly formulated with the idea of immunological networks or of idiotypic-anti-idiotype regulations (20–22).

2) Signal 1 + 2. Immune regulation is viewed as a binary system. It proposes that recognition of antigen by the immune receptors on T or B cells results in immune reactivity only in the presence of additional so-called second signals. In the absence of these signals, interaction with antigens results in silencing, i.e. anergy or deletion, of T or B cells (23–31).

We add a third proposal:

3) Antigen regulates immune responses. The localisation, dose and time of availability of antigen are the three essential dimensions determining whether an immune response is induced and for how long it lasts. This simple concept obviates the need for negative regulation, such as suppression or induction of anergy. It also dispenses the immune system with the need to be able to discriminate between self and non-self.

**Antigen localisation/dose/time regulating immunity**

What are the critical parameters that decide whether or not an immune response is elicited? Antigen dose and the duration of antigen presentation are of key importance for all three concepts of immune responses. We regard these two parameters as essential parts of a three-dimensional integral which is centered around the third and most important parameter, the geographical aspects of antigen, i.e. the role of antigen localisation in immunity.

Antigens that are permanently present in lymphoid organs, delete immune responses. Antigens that are permanently present within lymphoid organs and/or are continuously transported by migrating cells throughout the lymphohemopoietic system render the specific immune cells (T and B cells) unresponsive. For T cells this usually equals deletion, for B cells this is less clear. This simple rule applies to some self-encoded antigens (cell-associated and soluble self-antigens in the blood), and to foreign antigens if they reach organised lymphoid organs within a short period of time (i.e. 1–2 days) in an overwhelming manner and then persist. Human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), or subcutaneous or intravenous infection of slowly replicating LCMV do not usually fulfil this condition, because the initial rate of replication and spread is rather slow (i.e. >4 days).

Antigens that are always outside organised lymphoid tissues are immunologically ignored. Antigens that never reach organised lymphoid tissue or only for too short a period of time (i.e. less than 1–3 days) or at too low quantities cannot activate T cells. Again for B cells this is less clear. These antigens, be they encoded by the host or derived from external or infectious sources, are usually ignored immunologically (e.g. rabies virus in neurons or papilloma viruses in keratinocytes, or tumour cells of epithelial or mesenchymal origin in the periphery) (6, 32).

Rules for induction of an immune response. An immune response is induced if antigens enter the secondary lymphoid tissues in a localised manner, as defined earlier, and if antigen is present for at least some (>3–6) days. Such antigens usually include foreign antigens but may also include self-antigens that so far have
been immunologically ignored (category B), i.e. the antigen pool outside the organised lymphoid system.

**Experimental evidence to illustrate the geographical concept of immunity**

**Correlation between localisation of virus infection and immune responses**

Several viruses use the trick of staying outside lymphoid tissues to avoid immune surveillance. One example is Papilloma virus, which induces skin warts. This virus infects basal layer epithelial cells but replicates only in keratinocytes beyond the reach of Langerhans' cells, the APC of the skin (33). Similarly, rabies virus infecting neurons will be ignored by the immune system during the early phase of infection (34). Only when neurons are lytically destroyed will antigen be picked up by APC and transported to draining lymph nodes to induce a T- and B-cell response. While this process is often too slow to induce a protective immune response early enough to save the infected host, the induction of an immune response can be accelerated by forcing antigen into secondary lymphoid organs, i.e. by the post-exposure vaccination of Pasteur with attenuated or killed rabies virus. Because of the slow disease kinetics due to the neurotropism of rabies virus, vaccination can induce a protective immune response rapidly enough to catch up with the virus before too much damage by the cytopathic virus is done.

**Role of antigen transport to the draining lymph node in the induction of an immune response**

The skin-flap experiments. In 1957, Frey and Wenk (14) studied skin sensitisation on microsurgically isolated skin flaps of guinea pigs. These flaps were connected with the host via artery or vein. Contact sensitisation of the skin flap resulted in systemic immunisation of the guinea pig (as assessed by a rapid secondary response to a challenge on the rest of the host skin) only when the draining lymph vessel/nodes were left intact. If, however, the guinea pig was sensitised anywhere else, re-challenge via the skin flap revealed a prompt secondary reaction also without any intact lymph vessel or lymph node. In our modern understanding this experiment shows that APC with antigen must migrate via afferent lymph to local lymph nodes in order for T cells to be sensitised. Once T cells are induced, they recirculate and readily emigrate from the vasculature into peripheral solid tissue. This experiment was modified 10 years later by Barker & Billingham (35), who transplanted allogeneic skin onto the prepared skin flap of guinea pigs. They confirmed that for the induction of both a local rejection response and generalised priming, both the draining afferent lymph vessel plus the lymph node were critical. Once T cells were induced systemically, they could readily be recruited to the skin flap independent of afferent lymph and draining lymph node.

The Lafferty experiment. Additional evidence for this concept is provided by a classical experiment performed by Lafferty and co-workers (36, 37). Transplantation of allogeneic thyroid epithelial cells depleted of APC (passenger leukocytes) resulted in acceptance of the graft under the kidney capsule. Transfusion of donor APC 100 days after the transplantation, i.e. bringing antigen to organised lymphoid tissues, promptly initiated rejection (37). This experiment again demonstrates in our view that antigen which does not migrate into the draining lymph-nodes is ignored by the immune system.

The diabetes model with a transgenic antigen expressed in islet cells. Ohashi et al. (38) and, in a similar approach, Oldstone et al. (39), established a transgenic mouse model expressing LCMV glycoprotein (GP) under the rat insulin promoter (RIP). These RIP-GP-transgenic mice did not spontaneously develop diabetes. They did, however, promptly develop diabetes within 10 days of infection with the widely replicating wild type LCMV that induces a very potent LCMV-specific cytotoxic T-cell response. The induced effector T cells migrate to the β-islet cells in the pancreas expressing the transgen and destroy them. These experiments illustrate that T cells potentially reactive against self may be present, but because usually self-antigens do not reach organised lymphoid tissue in sufficient quantities and for a sufficiently long time to trigger many T cells, autoimmunity is not induced. This mouse model of CD8+ T-cell-induced autoimmunity revealed a second important result regarding the role of the quantitative immunological threshold for induction of autoimmune disease: infection of the RIP-GP-transgenic mice with a vaccinia recombinant virus expressing the LCMV-GP failed to induce diabetes although some infiltration (insulitis) was seen. Since infection with the vaccinia recombinant virus expressing LCMV-GP induces an LCMV-GP-specific CD8+ T-cell response that is about 100 to 1000 times weaker than that induced by an infection with LCMV wild type, this quantitative difference seems crucial for the induction of disease in this experimental model (40). Accordingly, it is not only important that autoreactive cytotoxic T cells are induced, but the quantity of the response is also important. This finding is relevant, because it underlines that the quantitative threshold for the induction of autoimmune disease is relatively high: only the widely spreading LCMV induces a sufficiently strong CTL activation to recruit them to the transgenic islets and to cause autoimmune disease (i.e. diabetes) in this model situation. As stated before, these threshold requirements are probably the
most important safeguard against T-cell-mediated autoimmune disease.

Experimental allergic encephalitis (EAE). Immunologically ignored self-antigen brought into organised lymphoid tissues in sufficient amounts for long enough time induces autoimmune disease (41). In EAE this is usually achieved by mixing the self-antigen, i.e. myelin basic protein with complete Freund’s adjuvant (CFA containing mycobacterium tuberculosis fragments) (42); the granuloma formed retains antigen for a prolonged time and promotes efficient presentation of self-antigen, particularly in the local lymph node (43). In addition, pertussis toxin is probably used to enhance the emigration of activated T cells to peripheral solid tissues, particularly to the brain.

Taken together, these four experiments show that T-cell immunity is not induced by antigen, either foreign or self, unless antigen reaches organised lymphoid tissue to a considerable extent and for quite some time.

Fibroblasts expressing a foreign antigen induce immune responses readily in lymphoid organs but not in the skin. Additional experiments in support of a simple geographical concept of cellular immunity were performed by Küntig et al. (44, 45). A methyl-cholanthrene-induced MC57G (H-2b) fibrosarcoma cell transfected with LCMV-GP was used as a model tumour antigen to study the induction of LCMV-GP-specific cytotoxic CD8+ T cells. Antigen-expressing cells were injected into the local lymph node or spleen or injected into the skin as peripheral solid organ. While about 10^5 to 10^6 cells had to be injected subcutaneously to prime syngeneic H-2b mice, about 5 x 10^4 (the lowest number tested) were still efficient in priming the GP-specific CTL response if cells were injected directly into the spleen. Detailed analyses showed that the fibroblasts themselves primed T cells and that LCMV-GP was not taken up and processed by host APCs, i.e. there was no cross-priming involved. These experiments demonstrated that fibroblasts that did not possess detectable co-stimulatory molecules and did not release co-stimulatory cytokines are capable of inducing effector T cells themselves. Again this only happens in the local lymph node or the spleen. This example suggests that a major reason for the failure to immunologically eliminate epithelial and mesenchymal tumours (which comprise > 80% of all malignant tumours in humans) is probably the fact that these tumours start growing in the periphery outside lymphoid tissue and are therefore ignored by T and B cells. In addition, warts illustrate the geographical aspect well; transformed virus-producing keratinocytes are beyond the reach of Langerhans’ cells of the skin. Therefore warts are non-immunogenic for variable periods of time until probably trauma promotes anti-

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1. Obviously, cytopathic viruses cannot overrun the immune system in this way, because they would kill the host too quickly for epidemiological success.
within 2–3 days, causes clonal deletion of the antigen-specific T cells (49, 50). The virus-specific immune response will remain deleted after infection if the virus persists and infects the thymus and deletes differentiating specific thymocytes. Thus while a potent CTL response is induced by an initially localised infection, the CTL response is exhausted if the infection is overwhelming.

Peptide antigen-mediated priming or exhaustion/deletion of cytotoxic T-cell responses is dependent upon distribution, dose and time. The parameters leading either to priming or tolerance of CD8+ cytotoxic T lymphocytes were examined with a major histocompatibility complex class I (H-2 Dd) binding peptide derived from the LCMV glycoprotein (GP aa33–41). The dose, route and frequency of LCMV-GP peptide application was varied. It was found that a single i.v. injection or a single local subcutaneous injection of 50–500 μg peptide without adjuvant failed to induce a measurable T-cell response or protection. If emulsified in incomplete Freund's adjuvant, a single subcutaneous dose of peptide protected mice against LCMV infection in a dose-dependent fashion. Repetitive and systemic intraperitoneal application of the same dose of peptide caused exhaustion/deletion of the T-cell response and tolerance of LCMV-specific T cells (51, 52). Such pretreated mice were not able to mount a peptide-specific T-cell response after infection with LCMV. The peptide-induced tolerance was transient in euthymic mice but permanent in thymectomised mice.

These examples illustrate the dose and time dependence of antigen localisation in the regulation of immune responses and underline the fact that it is the three-dimensional integral of all three parameters that determines whether protective immunity is induced.

Organised lymphoid structures in peripheral organs: reversed geography in some autoimmune diseases

Autoimmune and chronic immunopathological diseases are sometimes maintained by a reversal of the developed geographical principles. Instead of ignored self or new foreign antigen reaching and staying in organised lymphoid tissue, organised lymphoid tissue may be established in peripheral solid tissues, thereby providing conditions to maintain anti-self responses. A fascinating example is Hashimoto's autoimmune thyroiditis, where an inflammatory response against usually ignored self-antigen recruits lymphoid cells to thyroid tissue (16, 53). Within the infiltrated thyroid, the lymphoid structures are induced and the milieu is created which may maintain the response to local self-antigen. Although this is an overall rare event, these exceptions may serve to illustrate the principle that if lymphoid organ structures are newly induced in peripheral solid organs then the locally available antigens may maintain an abnormally induced immune response against usually ignored self-antigens.

Implications

The view that antigen alone drives immune responses has implications for autoimmune disease and for immunological memory. Let us restate the proposed rules: (1) Immunological unresponsiveness is maintained by deletion of T cells that recognise antigens that are always in the blood and/or presented appropriately by mobile cells present in organised lymphoid tissues. Such deletion occurs centrally during development in the thymus and/or in secondary lymphoid organs, and/or perhaps peripherally at a more mature stage of T cells (54–59). (2) Unresponsiveness due to indifference or ignorance is observed against antigens that usually do not exist in organised lymphoid tissues, including self-encoded and foreign antigens (60–62). (3) Induction of immune responses is caused by antigens that are either foreign and enter the host initially in a localised staggered fashion or by antigens which are encoded by the host and have so far been ignored, but now for various reasons are freshly brought to organised lymphoid tissues in sufficient quantities and for sufficiently long periods (63).

Relevance for autoimmune disease

The experimental conditions that induce and maintain autoimmunity have been discussed above for EAE or Hashimoto's thyroiditis. These examples may explain generally how autoimmune disease could arise. Accordingly host cells in peripheral solid organs may be destroyed to a sufficient extent and during a sufficiently long period of time by several mechanisms. Either cytopathic infections destroy host cells, or immunopathological-inflammatory mechanisms cause host cell destruction. Self-encoded antigens are thereby released, picked up by APCs and reach lymph nodes; alternatively self-antigens may reach the blood and get filtered out by the spleen. If such self-antigens have so far been ignored immunologically, these new conditions may reach critical levels of the integral determined by antigen localisation, amount and duration, that will result in an immune response to self-antigens.

Relevance for immunological memory

There is good evidence that a first exposure to an antigen will leave increased precursor frequencies of specific T and B cells in the host independently of whether antigen persists or not (64–66). However, without antigen persistence or periodical reappearance in lymphoid tissue, protective immune responses
disappear because T or B cells are not activated any longer (67, 68). Since, however, only activated B cells release antibodies to maintain elevated protective antibody levels in serum, protective antibody memory is antigen-dependent. Because antigen activates T cells and because only activated T cells can emigrate through peripheral solid tissue to control peripherally persisting infectious agents, protective immunological T-cell memory is also antigen-dependent. Again, as for T-cell induction, antigen persistence outside lymphoid tissue is not sufficient to maintain T-cell memory. It may, however, often serve as a source of antigen which periodically reaches lymphoid tissue to restimulate T cells. Similarly, the antigen-antibody complexes presented on follicular dendritic cells maintain B-cell memory by activating B cells to become plasmocytes that secrete protective antibodies. Possibly these depots may also be replenished from peripheral antigen sources and/or reinfec-
tion. Thus, while precursor frequencies of T and B cells are largely antigen-independent, antigens determine their activated status and thereby their in vivo protective capacity.

Implications for adoptive immunotherapy

The geographical concept presented also bears some consequences for adoptive immunotherapy with cytotoxic T cells (69, 70). If antigen localisation, dose and time of persistence determine the kinetics of the T-cell response, adoptive immunotherapy with T cells may sometimes be limited and unsuccessful for the following reasons.

If the persisting antigen is not present in host lymphoid organs, transfused immune T cells will not be restimulated and activated. Unless a very great number of highly activated T cells is transfused, they may stay in the lymphoid system and not emigrate in sufficient numbers to peripheral organs where viruses may hide in relatively few cells; therefore viruses only hiding in peripheral organs may not be reached by such cellular immunotherapy. In contrast, if antigen is localised throughout lymphoid tissue and widely spread through peripheral organs, transfused T cells may kill the host by generalised pathology or may be rapidly exhausted (71). Thus, observations made with adoptive transfers of CD8+ T cells suggest that a) they may in some cases eliminate the virus, or cause immunopathological disease - sometimes even death - of the host, or b) the T cells are exhausted and the virus persists, or c) they may ignore the virus in the periphery if they are not numerous and sufficiently activated exactly as has been discussed for the various antigen localisation, dose and time correlations in the previous sections. These considerations could explain the clinical observations that adoptive cellular immunotherapy against cytomegalovirus or Epstein-Barr virus may be efficient (69, 70), whereas expanded and activated tumour-infiltrating lymphocytes (TIL) may be less or not successful (72).

While these experiments illustrate the importance of geographical aspects for the induction of immune reactivity, the concept may probably also be extended to the effector phase. It is also likely that the effector function of T cells is maximal in lymphoid organs, where the chances of meeting the specific antigen are optimal when compared to the vast areas of peripheral solid tissues. Therefore it cannot come as a surprise that infectious agents or tumours (carcinomas or sarcomas) may escape immune surveillance in the periphery, even in a successfully primed host (Fig. 1c). Examples include cytomegalovirus (CMV) in kidney or lung epithelial cells, Herpes simplex virus in neurons of ganglia or, possibly, endogenous viruses in peripheral epithelial or neuroendocrine cells. Leishmania, other parasites or mycobacterium tuberculosis, i.e. infectious agents in granulomas in the periphery, also represent residual infections ignored by a primed immune response. These peripheral foci of infections serve as controlled sources of periodical antigen release to keep T cells activated; this is usually called protective T-cell memory, although operationally it is nothing else but a very low dose infection maintaining an ongoing immune response (67). This so-called infection immunity (73) is key to our understanding of the dose-response requirements of both immunological memory as well as of re-emerging infections in immunosuppressed conditions, i.e. in old age, HIV infections or during treatment with cytostatic drugs.

How do signal 1 and 2 models relate to the antigen localisation concept?

Signal 1 + 2 models postulate that in addition to antigen (signal 1), a second signal is required for the induction of immune responses. This second signal may be soluble as interleukins (e.g. IL-2) or may be cell-bound, e.g. CD28/B7. Organised lymphoid tissues provide the structures and the cellular milieu where all these factors are present in abundance and constitute an essential immunogenic environment. In this respect, our geographical concept incorporates second signals. We recognise that it may be difficult to distinguish between the role of localisation of antigen and the need for signal 2 in addition to antigen to induce immune responses. The reason for this difficulty is simply that the professional APC is the most important cell involved in antigen transport and in providing the second signal to T cells. However, we emphasise four important differences to previously suggested models (see below), which underline the notion that it is more important where antigen is localised than whether it is presented in the absence or presence of a particular signal 2. It should be noted
that these geographical requirements cannot be simulated in in vitro experiments, where the importance of the second signal is well documented for complex lymphocyte interactions.

The four important aspects of the geographical view of immunity compared with signal 2 models are:

1) There is no need for a particular signal 2 for the induction of protective immune responses. This is well illustrated by the relatively disappointing phenotypes of the various gene knock-out mice, which lack either IL-2 (74) or alternatively CD28 (75, 76), B7-1 (77), CD 40 or CD40-ligand (78–81). These gene knock-out mice exhibited virtually normal T- and B-cell induction and individual effector function. Contact-dependent cooperation and interactions were, however, often impaired. These findings suggest that second signals were of greater importance for specific contact-dependent interactions between lymphocytes, particularly T-B cooperation in immune responses against soluble antigen, but were not critical for T-cell response against intracellular infectious agents in lymphoid organs or for B-cell responses against maximally cross-linking antigens (i.e. paracrystalline ordered multiple identical determinants distant of 5–10 nm as present on virus envelopes, bacterial or parasite surfaces) (82–84).

2) Signal 2 given outside lymphoid tissue is of little biological relevance. As stated before, due to their recirculation pattern, the chances of naive T cells (which cannot emigrate into peripheral solid tissues) meeting their specific antigen on professional APC in peripheral solid organs is extremely low. In addition, in the absence of a lymphoid environment these interactions are inefficient and without biological consequence. Therefore, as a rule, T cells are not induced in the periphery.

3) The ability to induce immune responses is not bound to a particular cell type that can provide signal 2. As we have shown, antigen-specific induction of T cells is not an exclusive property of professional APC, since even fibroblasts can activate T cells, as long as they present the antigen within organised lymphoid tissues. The crucial role of APCs is therefore not defined by the fact that they express B7-1 or CD40 or release interleukins, but by their ability to transport antigen from the periphery to organised lymphoid tissue.

4) Induction of anergy is not necessary to assure peripheral unresponsiveness. It has been postulated that signal 1 in the absence of signal 2 results in anergy. As we have seen, there is no biological need for such an uneconomical silencing of immune responses, since antigen outside organised lymphoid tissue is simply ignored.

In conclusion, the geographical concept does not exclude the role for signal 2 and accessory molecules, but reduces them to hormone-like and contact-dependent survival or differentiation signals, rather than making them the deciders of what is self versus what is foreign, or of what is dangerous versus what is harmless.

References:


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