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Population Biology of Lymphocytes: The Flight for Survival

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Population Biology of Lymphocytes :

The Flight for Survival.

by

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Abstract :

In this essay we suggest that the primary goal of the cells of the immune system is to ensure their own growth and survival. In adults, in steady state conditions, the number and distribution of lymphocyte populations is under homeostatic control. New lymphocytes, which are continuously produced in primary and secondary lymphoid organs must compete with resident cells for survival. We discuss recent findings supporting lymphocyte survival as a continuous active process and implicating cognate receptor engagement as fundamental survival signals for both T and B lymphocytes. The conflict of survival interests between different cell types gives rise to a pattern of interactions, which mimics the behavior of complex ecological systems. In their flight for survival and in response to competition, lymphocytes use different survival signals occupying different ecological niches during cell differentiation. This is the case for T and B lymphocytes, and also for naive and memory/ activated T and B cells. We discuss how niche differentiation allows the co-existence of different cell types, and guarantees both repertoire diversity and efficient immune responses.

I. Introduction:

Life started by the arousal of self-replicating molecules (1). The first unicellular organisms emerged when these molecules developed the capacity to control their immediate surroundings to ensure survival and replication. With time, single cell individuals evolved to give rise to multicellular organisms. In these complex individuals, each cell is still imprinted with the same primordial program for self-replication and survival (2). An hierarchical organization is, however, established in which the survival and the rate of replication of the different cell types are restrained and their number controlled (3).

The individual cells of the immune system follow the same program, but they can only survive within the limited constraints imposed by the host. In adult mice the total number of lymphocytes remains constant and shows a "return tendency, due to a density dependent process to approach a stationary distribution of population densities" (4), usually referred to as homeostasis. T and B lymphocytes are, however, produced continuously in either the primary lymphoid organs or by peripheral cell division: it follows that each newly formed lymphocyte can only persist if another resident lymphocyte dies. In an immune system where the total number of cells is limited, cell survival can no longer be a passive phenomenon, but rather a continuous active process (5) where each lymphocyte must compete with other lymphocytes (6). It can be said that lymphocytes follow the Red Queen Hypothesis postulate "it takes all the running you can do to keep in the same place"(5, 7).

II. Lymphocyte Survival.

1. Naive T Cells.

Maintenance of naive T cells in peripheral lymphoid organs requires continuous T cell receptor (TCR) engagement by major histocompatibility complex (MHC) molecules. This was established for both $CD4^+$ and $CD8^+$ lymphocytes using various experimental approaches.

In the case of naive $CD8^+$ T cells, their capacity to survive was initially studied after adoptive transfer into mice expressing different MHC class I alleles. It was found that monoclonal naive $CD8^+$ cells from TCR transgenic (TCR-Tg) mice, restricted to MHC class I H-$2D^b$, survived without dividing when transferred to mice lacking H-$2K^b$, but expressing normal levels of H-$2D^b$ (8). In mice expressing lower levels of H-$2D^b$ only a fraction of the transferred cells survived – this fraction being proportional to the level of H-$2D^b$ expression. In H-$2K^{b+}Db^-$ host mice the TCR-Tg cells rapidly disappeared (8). The requirements for the peripheral survival of this naive $CD8^+$ T cell clone mimic, therefore, the requirements for positive selection of the same clone in the thymus (9), which include the continuous ligation of the TCR by MHC, the frequency of selected cells being related to the level of MHC class I expression (10). The existing experimental evidence indicates that the results obtained with this TCR-Tg clone apply to naive $CD8^+$ T cell populations in general. Naive monoclonal $CD8^+$ T cells, expressing a TCR-Tg specific for the gp33-41 peptide of LCMV and restricted to H-$2D^b$ (11) also persist after transfer into CD3ε-/ -H-$2K^b$-Db^+ hosts, but disappear by one week after injection into CD3ε-/ -H-$2K^{b+}Db^-$ hosts (Legrand & Freitas, unpublished). When wild type thymic epithelium was grafted into MHC class I deficient mice, mature $CD8^+$ T cell differentiation in the thymus was restored, but naive $CD8^+$ T cells produced in the thymus were unable to survive at the periphery (12).

Naive $CD4^+$ T cells also require allele-specific MHC class II interactions to survive (13, 14). Monoclonal TCR-Tg $CD4^+$ T cells, restricted to I-A^d, survive after transfer into I-A^{d+} recipients, but disappear after transfer into I-$A^{d-}A^{b+}$ recipients. Persistence of non-Tg $CD4^+$ policlonal populations also depends on interactions with MHC class II. Using retroviral vectors, class II expression was transiently induced in the thymus of class II-deficient mice allowing cohorts of $CD4^+$ T cells to be generated and exported into class II^- peripheral pools (15). Alternatively, MHC class II^+ fetal thymus grafts were implanted into class II deficient hosts (16). In both cases, permanent $CD4^+$ survival was dependent on the expression of class II at the periphery, but the exported $CD4^+$ T cells took 100 days to disappear in the absence of Class II. Although these results demonstrate that permanent mature $CD4^+$ T cell engraftment requires TCR/ MHC class II interactions, it appeared that in the absence of these interactions policlonal $CD4^+$ populations survived much longer than $CD8^+$ T cells. This relative prolonged survival could be due to the presence of activated cells; as most persistent survivor cells were of an activated phenotype (16) these cells (when compared to naive T cells) could be less dependent on MHC for survival (see bellow). It was also likely that within an heterogeneous policlonal T cell population some clones could interact with MHC class I or class II-like molecules, and thus be able to survive longer in absence of "bona fide" TCR/ class II interactions (17). Alternatively the prolonged $CD4^+$ survival could be due to the presence of MHC class II bearing dendritic cells (DC) exported by the class II^+ thymus (18). When a class II^+ thymus depleted of all BM derived class I^+ cells was grafted into class II^- mice, the $CD4^+$ T cells generated in the thymus did not survive at the periphery (18). When the transplanted class II-deficient hosts expressed a transgene of the same MHC class II molecule on the peripheral DC, the $CD4^+$ cells exported from

the thymus survived in the periphery (18). In this case $CD4^+$ T cells were shown to be in close contact with transgenic class II^+ DC, suggesting that this direct interaction plays a key role in $CD4^+$ survival. When these hosts expressed at the periphery a class II transgene of a different haplotype from the thymus class II, the majority of the naive $CD4^+$ T cells generated and exported from the thymus did not survive (T. Brocker, personal communication). These findings indicate that policlonal naive $CD4^+$ T cell populations require TCR/ MHC restricted interactions for peripheral survival. TCR/ MHC class II interactions are also required for the expansion of $CD4^+$ T cells in T cell deficient hosts (19).

Thymus positive selection requires the simultaneous recognition of MHC and peptide molecules presented by the MHC. The role of such peptide recognition in peripheral T cell survival was also investigated by comparing the fate of mature T cells in environments of restricted peptide complexity. In H-2Mα deficient mice the level of expression of MHC class II is not modified, but the loading of peptides into the MHC class II molecules is impaired. Most of the I-A molecules are occupied by the invariant chain peptide CLIP (20). This limited peptide display affects thymus selection and the peripheral T cells from these mice have a restricted repertoire diversity (20). It was reported that policlonal $CD4^+$ T cells and TCR-Tg cells restricted to $I\text{-}A^b$ do not survive when transferred either to H-2Mα deficient mice, to mice expressing a different MHC class II allotype or to MHC class II deficient hosts (14). The similar behavior of peripheral T cells in environments of restricted peptide diversity or MHC deficiency establishes that peptide recognition is essential for T cell survival. The peptides involved are probably similar to those mediating thymus positive selection. Thus, $CD4^+$ T cells from H-2Mα deficient mice which were selected by peptides expressed by the H-

2Mα deficient thymus survived when transferred into H-2Mα deficient peripheral pools but not when transferred into MHC class II deficient hosts (14). $CD4^+$ T cells selected by a single MHC class II/ peptide complex, survived without dividing in mice expressing the same complex at the periphery (21). These results demonstrate that, alike thymus positive selection, peptide recognition plays a role in peripheral T cell survival. They also suggest that the peptides involved in thymus positive selection and peripheral survival may be similar.

It thus appears that naive mature T cells, following emigration from the thymus, must continuously recognize MHC/ peptide complexes in order to survive in the periphery. Alike thymus positive selection this TCR ligation event is: a) MHC restricted, b) involves recognition of similar self-peptides, c) maintains bcl-2 levels of expression, since when TCR/ MHC interactions are discontinued bcl-2 levels decrease (13), d) does not induce extensive cell division as the vast majority of naive peripheral T cells appear to survive as resting T cells: CD44-TCR-Tg or wild type $CD8^+$ cells do not divide (8, 22, 23). Naive $CD4^+$ T cells do not divide or cycle very slowly (21, 24).

In spite of these similarities it is likely that peripheral survival and thymus positive selection signals are not overlapping. Positive selection is mainly determined by interactions between T cells and MHC/ peptide complexes expressed by the thymus epithelial cells, a cell type absent at the periphery. At the periphery a close interaction between surviving $CD4^+$ T cells with class II^+ DC was identified (18). It is not known if survival of naive $CD8^+$ T cells also needs interactions with particular cell types. Besides, thymus positive selection and naive T cell survival also seem to differ in lymphokine requirements as suggested by studies on H-Y antigen-specific TCR-Tg mice, deficient for the common γ chain of the IL-2 receptor (γc- mice). The γc-deficient mice have severe

defects on thymus T cell differentiation. As productive TCR rearrangements are rare, immature precursors survive poorly, most dying before TCR gene rearrangement. These abnormalities of thymus differentiation can be partially overcome when the γc- mice are crossed into an αβ-TCR-Tg background (25). Although producing $CD8^+$ mature T cells in the thymus, these mice still lack peripheral $CD8^+$ T cells. These results suggest that peripheral T cell survival is not solely dependent on signals mediated by the TCR, it may also depend on signals transmitted by lymphokine receptors. The lymphokine/ s required for naive T cell survival were not yet identified.

2. Memory T cells.

Memory T cells express at the cell surface a vast array receptors (lymphokine receptors, co-stimulatory and adhesion molecules), absent or expressed at low levels in naive cells (8, 26-28). It is likely that the expression of these receptors will modify the interactions of memory cells with their environment, in a yet unidentified way. Changes in overall receptor expression may contribute to modify the T cell avidity for antigen and/ or MHC, and to lower the thresholds of survival and activation (28-33). Moreover, some of these receptors may be able to transmit activation signals and induce cell division independently of TCR engagement.

The requirements for the survival and division of memory T cells are not yet completely characterized. Current studies, however, indicate that they differ from those of naive T cells, suggesting that lymphocytes are "able to tune their threshold of activation in response to recurrent signals" (34). Memory $CD8^+$ T cells expressing the H-Y specific TCR-Tg, restricted to MHC H-$2D^b$ class I survive and expand in the absence of either H-Y male antigen or the MHC restricting element in female H-$2K^{b+}D^{b-}$, but disappear in mice lacking both β2-microglobulin and H-$2D^b$ (presumably

expressing few MHC class I molecules) (8). The same anti H-Y TCR-Tg cells stimulated in vitro by the cognate peptide were transferred to TAP-1$^+$ Rag-deficient or TAP-1$^-$ Rag-deficient mice (35). All memory cells survived for at least 70 days in mice expressing TAP-1, while most Tg cells disappear two weeks after transfer into TAP-1 deficient recipients. To investigate if the behavior of this T cell clone can be extrapolated to CD8$^+$ memory T cell populations in general, we studied the survival of CD8$^+$CD44$^+$ policlonal memory cells from normal B6 mice. Since the MHC restriction elements of this policlonal population cannot be identified we followed their fate after transfer into syngeneic hosts lacking simultaneously β2-microglobulin, H-2D^b and H-2K^b MHC class I molecules. We found that one week after transfer 80-90% of the transferred memory T cells disappeared (Rocha, Freitas & Lemonnier, unpublished). Although long-term survival has yet to be studied, these results suggest that the vast majority of the memory CD8$^+$CD44$^+$ policlonal T cell populations still require some type of TCR/ MHC class I interactions to survive.

The requirements for the in vivo survival of memory CD4$^+$ T cells were not yet investigated, but probably they also differ from those of naive CD4$^+$ T cells. In MHC class II deficient mice the rare CD4$^+$ T cells that are able to persist at the periphery bear a very activated phenotype. Cells with a similar phenotype also persist in class II and β2-microglobulin deficient mice which appear to recognize MHC-like molecules (17).

Although memory T cell survival may require TCR-MHC-peptide interactions, in contrast to naive cells, this recognition does not appear to be MHC restricted (8). It is difficult to envisage how such non-restricted interactions would trigger memory T cells. Nonspecific signaling through the CD4/ CD8 co-receptors might be involved as Lck was found to be targeted to the CD8 co-receptor in LCMV specific memory CD8$^+$ T cells

(36). Alternatively a lower threshold of T cell activation would allow signaling by allo-MHC. It must be referred, however, that so far that memory T cell survival has been studied only after transfer into irradiated hosts. In these mice, memory T cells (but not naive cells) divide extensively (37). Part of this proliferation may be due to mitogenic factors liberated after irradiation. The crossing of MHC deficient mice into a CD3ε-/ - background will allow the study of memory T cell survival in intact hosts in absence of possible artifacts induced by irradiation.

Memory T cells also differ from naive cells in the state of activation associated with survival. While naive T cells persist mainly as long-lived resting cells, memory cell survival is accompanied by cell division (self-renewal). In normal mice a fraction of memory T cells are cycling and divide in the absence of nominal antigen stimulation (8, 23, 32, 35, 37-42). The rate of memory T cell division can be enhanced by the in vivo injection of lymphokines such as IL-2, IL-12, IL-15 and α-interferon (43). During immunization the increased production of growth factors also leads to the by-stander activation of memory T cells of unrelated specificity (31, 44). Memory T cell proliferation increases in conditions of T cell depletion. When mice are sub-lethally irradiated residual activated T cells expand (37). T cell expansion is evident when T lymphocytes are transferred to T cell deficient or irradiated hosts (38, 45). This expansion is at least partially responsible for the early increase in T cell numbers observed in treated AIDS patients (46) and in irradiated adults after BM transplantation (47). In these conditions peripheral mature T cells retain a considerable expansion potential which, in absence of intentional immunization, allows a single $CD4^+$ T cell to generate up to 10^{15} daughter cells upon sequential transfers into successive T cell depleted hosts (38).

The finding that memory T cells divide in the absence of their cognate antigen raises several (yet unanswered) questions. The first concerns the existence of long-lived resting memory T cells. It is possible that no memory T cell is actually in the Go phase of the cell cycle. Memory populations are likely not constituted by cells that cycle and cells that never divide. By studying the dilution of CFSE labeling in LCMV specific memory T cells in steady state conditions it was shown that all these antigen-specific memory T cells divide (Murali-Khrishna & Ahmed, in preparation). Studies of BrdU accumulation by memory $CD8^+$ T cells are also compatible with long cycle times and asynchronous division (23). If all memory cells are in some type of pre-activated state this may favor their rapid and efficient engagement in secondary responses (Veiga-Fernandes & Rocha, submitted). The second question refers to the type of signals driving antigen-independent division. Survival and proliferation of memory cells may be driven by IL-15 (48) or by several other cytokines which are able to increase the rate of proliferation of memory T cells (43). It is probable, however, that some type of cognate TCR/ MHC/ peptide interactions may also be involved: $CD4^+$ cells selected by a single MHC/ peptide complex do not divide after transfer to irradiated mice expressing the same complex, but expand after transfer into normal mice (21). These results suggest that peptides responsible for thymus positive selection and peripheral survival differ from those driving peripheral T cell division, the latter phenomenon involving some type of TCR "cross"-reactivity.

Finally the most important question concerns the functional activity induced by "non-specific" T cell stimulation. What are the functional differences between these "naturally" activated T cells and T cells triggered in the course of an immune response? Several (non-exclusive) possibilities may be considered. Low affinity signals may 1)

reduce the frequency of the cells engaged in different effector functions, or 2) reduce the efficiency of each different effector function. For example production of cytokines after "non-specific" stimulation may be so low that they can only promote autocrine growth (and maintain antigen-independent division of memory T cells) and would be unable to act on neighboring cells. 3) Different effector functions may require different thresholds of activation. It can be envisaged that some type of cytokine secretion (required for memory T cell growth) can be induced by "weak" interactions, while T cell cytotoxicity depends on a "strong" T cell activation. In a lymphocyte receiving multiple signals through several cell surface receptors what will determine the "strength" of T cell activation? How lymphocytes integrate TCR-mediated signals with signals transmitted by other cell surface molecules is largely unknown. These signals may act independently, antigen-specific engagement being absolutely required to induce some effector functions. Alternatively some type of integration may take place: in this latter case a "non-specific" TCR engagement associated with other intense stimulation through other receptors may also be able to induce "strong" T cell activation.

The answer to these questions has major implications to our understanding of auto-immunity. Mature memory T cells are continuously triggered by "non-specific" TCR/ MHC/ peptide engagement. Strong activation through alternative receptors may explain the emergency of auto-aggression in conditions of lymphokine imbalance (49, 50), or deficient control of T cell activation and growth (51, 52). Secondly, situations in which T cell repertoires are restricted are now frequent. In AIDS or after irradiation or chemotherapy the restoration of immune-competence may be dependent of our capacity to induce "low affinity" T cells to mediate effector functions.

3. B cell survival.

Survival of naive B cells in the peripheral pools also appears to involve interactions between the B cell receptor (BCR) and yet unidentified ligand(s). This was first suggested by experiments in which a transgenic BCR could be ablated by an inducible Cre-LoxP recombination event. It was reported that after BCR ablation, B cells rapidly disappeared from the peripheral pools (53). This study, however, did not allow to directly correlate BCR signaling and peripheral B cell survival, as BCR ablation also leads to the arrest of new B cell production in the BM (54). In the absence of the newly formed BM migrants a significant fraction of the peripheral B cells is rapidly lost (55). In mice with a deletion mutation of the Igα cytoplasmic tail, early B cell development in the BM exhibits only a small impairment but the generation of the peripheral B cell pool was severely reduced (56). The question remained on whether the mere presence of a signaling complex, e.g. IgM-Igα-Igβ or other, at the cell surface suffices to signal B cell survival, or if B cell survival requires ligand recognition. Other studies addressed directly the role of ligand-mediated recognition in peripheral B cell survival. B cells lacking the V-region of the IgM receptor were shown to have a very short life-expectancy (57). The presence of the truncated membrane IgM transgene, lacking the V-region, provides constitutive signals that suffice to signal allelic exclusion and to promote pre-B cell development in the absence of the surrogate light chain λ5 (58), but fails to support long-term survival of the Tg B cells (57). Differences in the antibody repertoires expressed by pre-B and peripheral B cells can also be invoked to suggest the involvement of ligand-mediated recognition in peripheral B cell positive selection and persistence (59-61).

In contrast to T cells in which TCR survival signaling seems to require the recognition of MHC class I or class II molecules, the nature of the ligand(s) that might

be involved in B cell survival remain elusive. Analysis of V_H-gene family expression has provided evidence for a very conserved pattern of V_H-gene family usage that is independent of the V_H-gene number, is strain and tissue specific and is tightly regulated during B cell differentiation (60). After B cell generation in the BM, naive B cell survival is associated with a strong peripheral selection of B cells expressing particular V_H-gene families (61, 62). These observations suggest that the recognition interactions related to B cell survival may not require the involvement of the full antigen-binding site and might be exclusively V_H-mediated. It is possible that this type of ligand recognition may not lead to full cell activation and that low avidity interactions suffice to promote cell survival. It was recently shown that the BCR is capable of differential signaling and that B cell responses may differ depending upon the properties of the antigen. Thus, while some B cell responses were better correlated with antigen-BCR affinity than with receptor occupancy, other were only weakly dependent on antigen affinity (63).

Memory B cells not only show phenotypic changes and express an hypermutated BCR of a different isotype with an increased avidity for antigen, but show also an higher rate of cell division, a lower threshold of activation and occupy a different habitat within the secondary lymphoid organs. Studying memory responses to both thymus-dependent and independent antigens and using different cell transfer systems, it has been claimed that long-term persistence of B cell memory requires continuous cell division in the presence of antigen (64-66). Recent observations in mice in which an inducible Cre-loxP-mediated gene inversion (67) is used to change the specificity of the BCR contradict these results. In these experiments it was shown that after antigenic stimulation and the generation of memory B cells, memory cells survived even after expressing a new BCR unable to bind to the original activating antigen (Maruyama,

Lam and Rajewsky, personal communication). These findings suggest that antigen may only be required at early steps of cell activation and selection of high affinity B cells in the germinal centers. Once these B cells acquire a "memory phenotype", they may no longer require antigen recognition for long-term survival.

In this context it is important to refer that the difference in the interactions required for the survival of B and T cells may explain the different rate of mutation of B and T cell receptors. Since T cell survival requires recognition of ubiquitous MHC molecules, somatic mutation of the TCR might always be disadvantageous as it may cause loss of MHC recognition and thus cell death. In contrast somatic mutation may increase the BCR-ligand avidity and favor selection of high affinity B cells which, compared to the initial population of non-mutated B cells, have a competitive survival advantage within the germinal center. In their flight for survival, B cells are still able to use mechanisms of receptor editing (68-70) and change BCR specificity to escape cell death and may be gain a survival advantage over other rival cells.

Lymphocyte survival is likely to involve multiple mechanisms. Different signals may engage different cell surface receptors using the same or different survival pathways. Naive T cell survival depends on signals transmitted via lymphokine receptors (71) and it requires the expression of LKLF (72) or NFAT4 (73) transcription factors. The constitutive expression of the Ebstein-Barr virus LMP2A protein in transgenic mice bypasses BCR signaling and allows the survival of receptor-less B cells in the peripheral pools (74). Downstream of the BCR signaling pathway defects in CD45 (75) ,Btk (76), Syk (77), or NF-κB (78) or in the OBF-1 transcription factor (79) also affect peripheral B cell maintenance. Lymphocyte survival is also modified through the balance between different apoptotic and anti-apoptotic proteins (80-82). It has been

shown that BCR signaling and increased levels of intracellular bcl-2 ensure lymphocyte survival through independent pathways (53, 57). It is possible that the expression of bcl-2 may simply increase cell efficiency by lowering the threshold of resource requirements for survival.

III. Homeostasis and Competition.

1. Lymphocyte production.

In adult mice the potential to produce new lymphocytes in the primary lymphoid organs or by peripheral cell division, largely exceeds the number required to replenish the peripheral pools.

Firstly, the number of immature T cell precursors in the thymus is not limiting. A normal sized T cell pool can be generated in Rag2-deficient mice reconstituted with a mixture of 50% normal BM and 50% BM cells from a CD3ε-/ - deficient mice which are unable to generate T cells. The thymus production of mature T cells is also in excess as shown in mice in which thymus export is artificially reduced. Mice with reduced thymus export are Rag2-deficient hosts reconstituted with normal BM cells diluted in TCRα deficient BM (that can not generate mature T cells). In these mice peripheral T cell numbers are maintained in spite of the lower production of mature T cells (A. Almeida & Freitas, unpublished). Finally, T cells can also be produced by peripheral cell division. In a normal mouse peripheral division is restricted. When peripheral T cells are transferred into a T cell deficient host they are able of considerable expansion (38). It was found that each lymphocyte could generate a progeny of about 10^{15} cells (38). Therefore a single T cell might be sufficient to repopulate the peripheral T cell pool of a mouse. Secondly, an increase in lymphocyte production is not directly reflected in the size of the peripheral T cell pool. Mice grafted with increasing numbers of thymuses do

not show a proportional increase in the total number of naive peripheral T cells (83-85). All these results indicate that the total number of peripheral T cells is not necessarily determined by T cell production, but is limited at the periphery.

Mature B cells are produced in much higher numbers than those required to fill the peripheral B cell pool. The number of B cell precursors is not limiting as shown in mice in which the B cell precursor number is artificially reduced. The number of pre-B cells can be reduced in Rag2-deficient mice which were lethally irradiated and reconstituted with mixtures of normal BM cells diluted among incompetent BM cells from B cell-deficient (μMT) donors. In these chimeras the number of BM pre-B cells is proportional to the fraction of normal BM cells injected. Mice containing less than 25% of the normal number of pre-B cells had reduced peripheral B cell numbers. A normal sized peripheral B cell pool, however, was generated in mice containing only 30% of the normal number of pre-B cells. These results demonstrate that about 1/ 3 of the normal number of BM B cell precursors suffices to maintain the peripheral B cell pool size (86). A similar conclusion was obtained after parabiosis between one normal and two or three B cell deficient mice. In these circumstances B cell production was restricted to the BM of the normal mouse since no chimerism was detectable in the BM of the different partners. In mice triads it was found that each individual mouse had physiological B cell numbers i.e. the B cell production of one mouse was sufficient the populate the peripheral pools of three mice (86). These results demonstrate that peripheral B cells number is not determined by the rates of BM B cell production, but it is limited at the periphery.

2. Demonstration of Lymphocyte Competition.

In an immune system where new lymphocytes are continuously produced in excess but their total numbers are kept constant, newly generated cells have to compete with other newly produced or resident cells to survive. Competition can be defined as "an interaction between two populations, in which, for each, the birth rates are depressed or the death rates increased by the presence of the other population"(87). There are two main established criteria accepted as evidence of competition among populations: 1. The presence of competitors should modify the equilibrium size of a population and 2. should alter the dynamics, e.g. the life-span of a population (88). The question of whether competition arises between B and T cells was addressed by comparing the development and the fate of TCR or BCR-Tg and non-Tg populations in several different lines of mouse BM chimeras (89, 90). It was found that: a) when injected alone, Tg and non-Tg cell populations show an identical behavior and generate peripheral pools of similar size. b) when Tg and non-Tg cells are mixed in the same host they initially accumulate at the same rate. However, after reaching steady state numbers there is a preferential selection of the non-Tg cells at the periphery. These observations fulfil the first criteria for competition since they demonstrate that the presence of non-Tg populations modifies the number of the Tg cells (89, 90). In these experiments it was also found that that the life-expectancy of the Tg T or B cells varied according to the presence and the type of other competing cells (89-91). These latter findings fulfil the second main criteria required for the definition of competition as they prove that the presence of competitors alters the life-span of a population.

By considering each cell clone as a species, competition between Tg and non-Tg cells represents an example of inter-specific competition. Competition may also occur between individuals of the same species (intra-specific competition) (87). Studies using

monoclonal mice may allow to determine whether competition also occurs among cells of the same clone.

3. Types of Competition and Resources.

Competition may arise through different processes. In interference competition populations may interact directly with each other or one population can prevent a second population from occupying an habitat and from exploiting the resources in it. Thus, although interference competition may occur for a resource it is "only loosely related to the resource level" (87). In exploitation competition different populations have a common need for resources present in limited supply. In this case competition is directly related to level of resources available.

We may define "resource" as any factor which can lead to increased cell survival or growth through at least some range of their availability (88). On a broad sense a resource is any factor which is "used" by a cell and for that reason is no longer available to other cells. Resources can be essential, complementary, substitutable, antagonistic or even inhibitory (92).

There is ample evidence for the role of resources in lymphocyte competition. 1) The kinetics of accumulation of Tg and non-Tg populations after reconstitution in different groups of mouse chimeras followed a density dependent growth curve which follows the Monod growth function, i.e. "it increases in a saturating manner with resource availability" (93). During the expansion phase of cell reconstitution, resources are abundant and both Tg and policlonal populations accumulate at the same rate. When population growth reached equilibrium, policlonal populations become dominant, i.e. competition only occurs when resources are limiting (89, 90). 2) As discussed bellow changes in resource level should alter the balance between

populations. Antigenic administration to chimeras hosting different Tg cell populations favors the dominance by the antigen-specific Tg cells (91, 94). 3) Experiments demonstrating that the total numbers of peripheral T and B cells are not determined by rates of new cell production, but are limited at the periphery, represent further evidence to the existence of resource competition.

In the immune system many molecules can function as resources, e.g. antigen, MHC molecules, ligands for co-stimulatory and adhesion molecules, mitogens, interleukins, chemokines, hormones and other growth factors, etc. Resources can be external to the immune system or be produced by the lymphocytes themselves. By producing their own resources lymphocytes also contribute to generate their own ecological "space".

4. Resource Availability Shapes Lymphocyte Populations.

Resource competition may be determinant to the regulation of the size of peripheral lymphocyte pools (94); if peripheral resources are plenty many more newly generated T and B cells are able to survive (86). Any manipulation of resources may modify lymphocyte populations.

When resources are used by many cell types, changes in these common resources will modify overall cell numbers. Hormones and mitogens are examples of pleiotropic resources. The size of T and B cell pools is diminished in mice deficient in growth hormone and is increased in adrenalectomized, ovarectomized, castrated, pseudo-pregnant and pregnant mice (Reviewed in (6)). In axenic mice lymphocyte numbers are reduced and lymphocytes expressing activation or memory markers are rare or absent (Rocha, unpublished). Normal sized peripheral pools are rapidly restored as soon as the

normal gut flora is reestablished or after its colonization by a single fungus or bacteria species.

Activated lymphocytes and antigen presenting cells (APC) are major producers of their own resources. Antigen stimulation increases resource availability by inducing macrophage and lymphocyte activation and the production of numerous cytokines. In this context, lymphocytes can increase in numbers as during the expansion phase of the immune response (95). Once antigen is eliminated cytokine production decreases. Reduced resource levels are unable to maintain the same number of lymphocytes: most will die during the contraction phase of the immune response (95). Antigen can also be considered as an example of a private resource, i.e., that used by a particular cell set. Changes in the levels of private resources modify the composition of lymphocyte populations, as when antigen injection to chimeras carrying mixtures of two different Tg B cells or of Tg and non-Tg B cells shifted the balance between populations to favor the antigen-specific Tg B cells (91, 94).

In conditions of resource competition one would also expect changes in "morphology" of a population in response to the presence of competitors: a process known as "character displacement" (96). The IgM secreted into the serum by a population of normal B cells exhibits different binding patterns according to the presence or absence of a population of Tg B cells (94). This implies that the presence of the Tg B cells leads to changes in the selection of the non-Tg B cell clones, a process that at a population level may be considered to mimic character displacement.

5. The Competition Exclusion/Diversity Paradox.

According to the competition exclusion principle, competition should lead to the progressive dominance of a limited number of clones and consequently to the exclusion

of most clones (87). In this context lymphocyte competition would be incompatible with maintenance of diversity. This traditional model of population dynamics assumes that all cells interact equally well with each other in small closed environments. The more recent metapopulation approach takes into account difference in the age and structure of the populations, their migration capacity, the heterogeneity and patchwork distribution of fragment habitats and their change with time (4, 97). This approach explains the co-existence of multiple potentially competing species within larger areas of space. According to this theory, there are several mechanisms through which the immune system can solve the apparent competition-diversity paradox and select preferentially for diversity.

1. Continuous cell production. The continuous generation of naive cells ensures the permanent availability of new cell specificities. The contribution of new cell production to the maintenance of diversity can be evaluated by comparing lymphocyte repertoires in young and old individuals. With age the progressive decrease in new lymphocyte production both in the thymus and in the bone-marrow generally correlates with an increased frequency of pauciclonal repertoires.

2. Terminal differentiation. Control of cell proliferation by terminal differentiation prevents unlimited expansion and dominance (34). This is the case of B cell terminal differentiation into Ig-secreting plasma cells.

3. The redundancy of resources. The capacity to use multiple alternative factors for survival and proliferation may provide a critical advantage for cells competing for limiting resources. Indeed, it was shown that B cells from a LPS-reactive mouse strain have a clear competitive advantage when compared to B cells from a non-LPS reactive strain (89).

4. Diversification in resource usage. The use of different resources by different populations of lymphocytes will allow the co-existence of different cell types. Cell differentiation is associated with the acquisition of receptors for different chemokines, and growth factors or by regulation of homing receptors. These parameters contribute to create a heterogeneity of habitats that favors co-existence of diverse potentially competing cell populations (4). Migration ensures the distribution of cells from source to the periphery and between the different peripheral environments (98): cell survival is associated with the adaptation to new ecological niches.

6. The Ecological Niche.

The variety of resources and the heterogeneity of anatomical structures in the lymphoid organs permits that different lymphocyte types may find an ecological niche to survive. The inability of the cell to migrate into the niche may compromise survival: exclusion of B cells from follicular niches in the secondary lymphoid organs has been claimed to result in cell death (99). Niches may also play a role in homeostasis and in the control cell numbers by forcing the B cells to migrate into the primary follicles (100) where they must compete for trophic factors present in limited supply.

Lymphocytes may help to define their appropriate niches. Thus differentiation of epithelial cells in the thymus and the organization of the thymus cortex and medulla cellular networks depends on the presence of thymocytes (101); the maturation of the splenic follicular dendritic cell networks and the organization of B cell follicles depends on the expression of LTα and β and TNF by B lymphocytes (102-104).

Germinal centers that develop in the B cell follicles during T cell dependent antibody responses are one of the best characterized ecological niche in immunology (105-107). Germinal centers are oligoclonal; the B cells that give rise to germinal centers

are initially activated outside follicles and on the average three B cells colonize each follicle. Massive clonal expansion of the initial founder cells, driven by antigen held by follicular dendritic cells, prevents colonization of the germinal center by B cells specific for a second unrelated antigen. Expansion of the B cells is accompanied by BCR hypermutation. Competition among the proliferating B cells based on their ability to interact with antigen held on FDC, will lead to the preferential survival of the cells capable of high affinity recognition and to the death by apoptosis of other cells. Germinal centers play therefore a critical role in the maturation of immune responses by selecting cells with high avidity for antigen binding. They seem to have evolved to provide the appropriate niche for selecting B cells which attempt to gain competitive advantage for survival by somatic hypermutation. Indeed, somatic hypermutation is present in philogeny (108) well before the development of the affinity maturation of immune responses (109). Affinity maturation of the immune response, however, is only present in species capable of germinal center formation.

When heterogeneous populations occupy heterogeneous habitats the probability of extinction of a species is higher for the rare populations (97). It is possible that the same rules may apply to the immune system. In mice arrest of new B cell production leads to preferential extinction of B cells expressing rare V genes (61). The probability of extinction of a population is also related to the area of habitat occupied. Destruction of wide areas of habitat can be predicted to be more damaging for the survival of the most frequent species with a wider distribution than for some rare species which commonly occupy restricted niches and may therefore escape catastrophic events. Large structural changes in secondary lymphoid organs (e.g. sub-lethal irradiation) have been shown to affect predominantly dominant B cell clonal responses (110).

IV. Niche Differentiation.

1. Different Types of Lymphocytes Occupy Different Niches.

Different lymphocyte subsets likely use different resources. In adult mice the size of T and B lymphocyte populations is independently regulated. The number of mature B cells is similar in normal, or in mice which lack T cells (111). In mice that lack B cells, the number of T cells is similar to that of normal mice (54). B and T cells also occupy different locations in the secondary lymphoid organs (112, 113). These observations suggest that B and T cells belong to different guilds, i.e. they exploit different resources. Among the T cell subsets the number of $\alpha\beta$ and $\gamma\delta$ T cells is also independently regulated (114): in the absence of $\alpha\beta$ T cells, the number of $\gamma\delta$ T cells does not increase significantly and vice-versa. Within $\alpha\beta$ T lymphocytes the number of $CD4^+$ and $CD8^+$ T cells is, however, co-regulated (38). In the absence of either of the two $CD4^+$ or $CD8^+$ T cell subsets, cell loss can be compensated by the remaining cellular subset and the total number of $\alpha\beta$ T cells remains similar to that of normal mice (115-117) suggesting that $CD4^+$ and $CD8^+$ T cells may partially share common resources.

2. Naive and Memory/Activated Cells Belong to Different Niches.

In response to the presence of competitors a population may modify its survival requirements and adapt to a new ecological niche.

During lymphocyte development, cells bearing the same clonal specificity but at different stages of differentiation (immature vs. mature; naive vs. memory; Th1 vs. Th2) modify either their use of resources or interact differently with the same resources. The variety and the patchy distribution of resources in different habitats will make that the adaptive lymphocyte environment will change according to time and tissue

localization. Lymphocytes were shown to engage in niche differentiation as it is the case for naive and activated/ memory populations of cells.

The peripheral pool of $CD8^+$ T cells was shown to be divided in two compartments (naive and memory) of equivalent size, that are regulated by independent homeostatic mechanisms. Mice manipulated to contain only naive $CD8^+$ T cells, in spite of available "space", have only half of the total number of $CD8^+$ T cells. Similarly, in mice engineered to contain only memory $CD8^+$ T cells, the total number of $CD8^+$ T cells is also reduced by half (37, 118). To reconstitute a normal sized compartment, both naive and memory T cells are required. The existence of independent niches for naive and memory T cells ensures the coexistence of both cell types, what has major functional implications. Thus, the continuous thymus output of naive T cells cannot lead to the extinction of previously generated memory responses as exported naive thymus migrants do not replace resident memory T cells (119). Survival of naive T cells relies on the nature and the number of other competing naive T cells (90, 119). Conversely, accumulation of memory cells will not lead to the contraction of the naive T cell pool: successive antigen encounters will not necessarily result in pauciclonal repertoires, as the diverse naive repertoire will remain. Resident memory T cells survival will rather be determined by the expansion of newly generated memory T cell populations: upon the sequential immunization of mice with different types of virus (120), response to a second antigen leads to the reduction of the residual memory to a first antigen. It is not yet known if $CD4^+$ T cells follow the same strategy, but preliminary results suggest that this may be the case (A. Almeida & Freitas, unpublished).

In the case of B lymphocyte populations the number of resting and activated IgM-secreting cells is also independently regulated (86, 121), as shown in mice engineered to contain different numbers of B cells. In mice with very low numbers, a significant fraction of the B cells shows an activated phenotype and the number of IgM-secreting cells and serum IgM levels are as in normal mice (86, 121, 122). Mature resting B cells only accumulate once the activated pool is replenished (121). These results suggest that the immune system is organized to first ensure the maintenance of normal levels of natural antibodies ("innate immunity") which constitute an initial barrier of protection while keeping a reserve of diversity among the resting B cell compartment that allows responses to new antigenic stimuli. The independent homeostatic regulation of resting and activated/ memory lymphocyte compartments implies the existence of an hierarchical organization of the immune system.

3. Lymphocyte Substitution.

From birth to adult life, lymphocyte pools increase in size and can accommodate newly generated lymphocytes. The situation differs once steady state numbers are reached, the "space" is full: newly generated lymphocytes survival will be determined by the rate of cell substitution.

Substitution is conditioned by the rate of colonization (invasion) by new cells, but it is also determined by the rate of extinction of pre-established cells. To survive a cell must migrate and find the appropriate niche. If the niche is empty there is no obstacle to cell entering. If the niche is already colonized the newly arriving cell is either able to out-compete and displace the established cell or it is prevented from entering the niche and dies. A population pre-established in the correct niche and with a low extinction

rate benefits from the founder advantage "first come, first served". It may resist replacement by a new coming cell.

The most extreme case of substitution is succession in which the vast majority of a resident population is replaced by a population of newly arriving cells. This situation probably occurs during ontogeny, as lymphocytes produced during the perinatal period are replaced by "adult" cells. Thus, T lymphocytes generated early in ontogeny perform poorly when compared to adult T cells (Reviewed in (123)). They proliferate poorly and secrete low levels of certain cytokines in response to in vitro anti-TCR stimulation. In vivo proliferation in response to anti-TCR or antigenic stimulation is also impaired (124, 125). This is due both to an intrinsic lymphocyte defect, and to deficient antigen presenting cell function (125). These characteristics of the perinatal environment are believed to be the basis for its increased susceptibility to tolerance induction (126). Affinity maturation may be absent, as suggested by the extensive cross-reactivity of neonatal T cells repertoires (127).

Similarly, neonatal B cell repertoires differ from those of adult mice. In neonatal mice the D_H proximal V_H7183 gene family is used at the same frequency by 30% of the immature pre-B cells in the BM and 30% of the peripheral B cells (60), while in adult mice B cells expressing V_H-genes of the V_H7183 gene family are strongly counter-selected at the pre-B to B cell transition (62). By comparing the binding specificities of B cell hybridomas it was also found that the vast majority of the antibodies were multi-reactive in neonatal mice, (128), while in adult mice most of the antibodies were mono-specific (129). These changes of B cell repertoires correlate with overall modifications of B cell dynamics (55, 130) suggesting that B cell selection is permissive in neonatal mice during the phase of expansion of lymphocyte numbers while it becomes strict in adult

mice when resources become scarce and competition is established. The replacement of both T and B cell neonatal cross-reactive repertoires suggest that in ontogeny lymphocyte populations go through a process of ecological succession in which generalist cells are replaced by specialists.

Analysis of the processes of succession also may reveal other types of interactions between populations (97). The presence of an early population by modifying the environment may prepare the site for colonization by a latter cell type, a process known as facilitation. Examples of these should be looked for in the immune system.

How does substitution works in the adult mice? Studies of the substitution of resident naive T cells by recent thymus migrants gave contradictory results. In irradiated TCR-Tg mice injected with normal BM cells, substitution of the naive Tg $CD8^+$ T cells by newly generated naive non-Tg cells was found to be age-independent e.g. thymus migrants and resident cells appeared to have the same survival probability at the periphery (119). Opposite results were obtained in mice transplanted with increased number of fetal thymus grafts. These grafts initially exported fetal T cell of donor origin, but where subsequently colonized by host BM cells. The study of the substitution of T cells of graft origin by T cells of host origin suggested that recent thymus migrants survived better than resident naive T cells (85). Finally, the study of long-term colonization and persistence of recent thymus migrants labeled in vivo with CFSE seems to suggest the resistance of resident cells to replacement by the newly arriving cells (131). Similar conclusions were made from parabiosis experiments (90). The reasons for these conflicting results no doubt derive from the complexity of the different experimental systems required to study substitution. All experimental approaches involved stressful conditions such as irradiation or surgery. Very diverse

populations (Tg vs. policlonal; fetal vs. adult; etc.) were compared. Finally, conclusions on substitution rates rely on the quantification of very low T cell numbers. The definite answer to the this question requires new yet unavailable experimental approaches.

As naive and memory T cells belong to different niches (see page 21), naive recent thymus migrants do not substitute resident memory T cells (119). Surprisingly, thymus migrants are able to dislodge resident tolerant T cells. Tolerant $CD8^+$ Tg cells can persist at high frequencies for prolonged periods of time in then absence of other $CD8^+$ T cells (132). Tolerant Tg $CD8^+$ T cells can be substituted by both thymus migrants (119) and memory $CD8^+$ T cells (Rocha, unpublished). This replacement is such that a significant part of these cells disappear, but a few cells remain for prolonged time periods. The residual tolerant Tg T cells are unable to eliminate antigen but are not inert. They secret IL-10 and γINF (133) and may also play a role in the control of immune responses by interference competition (134, 135).

In the naive B cell pool substitution of naive B cells by B cells produced in the BM occurs continuously. Maintenance of the physiological size of the peripheral B cell pool requires a minimal continuous input of new cells, since mouse chimeras with a 10 fold reduced B cell production show diminished B cell numbers (86). Abrogation of the "de novo" B cell production leads to a rapid decrease in naive B cell numbers (55, 136). The excess of B cell production in normal physiological conditions suggests the existence of an high attrition rate at the periphery (55). Tolerant B cells from double-transgenic mice co-expressing hen egg lysozyme (HEL) and anti-HEL Ig-genes have a relatively short life span when compared to normal B cells (137).

Naturally activated IgM-secreting B cells can resist replacement by newly arriving B cells. They can also prevent the entry of new emigrating B cells into the activated cell

pool (121). This negative feedback regulation may be due to the secretion of inhibitory factors, e.g. Igs, IL-10, γ-INF. Alternatively, the first established population may occupy a niche required for the selection and survival of incoming cells preventing colonization (87). The ability of newly formed B cells to differentiate into IgM plasma cell is dependent on the nature and number of cells already present at the periphery. Early during development and expansion of the immune system an initial pool of activated B cells is selected which may resist replacement. The same IgM-secreting cells are also found in axenic mice, suggesting their activation by "self-antigens". Recent evidence suggest a possible role of self-antigen in the positive selection and activation of auto-reactive B cells (138). Competition based on the BCR diversity and the antigenic environment heterogeneity eventually leads to the substitution of the initially selected population by new specificities formed in the BM. The question remains on the possible functional differences between these "naturally" activated B cells and the B cells triggered in the course of an immune response.

V. Mutualism.

Besides competition, populations of cells also interact differently. One of the most important types of interactions is cooperation, also called mutualism. Through cooperation the survival and growth rate of one population is increased in the presence of a second population. During evolution eucaryotic cells represent the first example of mutualism (139). Multicellular organisms, where no cell survives on its own, are yet another example of mutualism.

There are multiple examples of mutualism in the immune system. T and B lymphocytes evolved mutualistic interactions: $CD4^+$ T cells help proliferation and differentiation of antigen-specific B cells. Reciprocally, antigen presentation by B cells

contributes to the expansion of antigen-specific T cells. $CD4^+$ T cells enhance cytolytic $CD8^+$ T cell responses. Similarly, T lymphocytes and antigen presenting cells (APC) have evolved mutualistic interactions, essential for T cell survival and for triggering immune responses. Dendritic and other APC may therefore play a keystone role in the establishment of lymphocyte communities.

Predator-prey interactions may also shape lymphocyte populations. In chimeras reconstituted with mixed populations of BM cells from male and female donors, the immediate injection of TCR Tg $CD8^+$ T cells specific for the HY male antigen leads to the elimination of all cells from male origin. This is followed by a subsequent increase in the number of cells from the female progeny (132). In fact, in this example the fate of the two BM cell types mimics the dynamic behavior of two competing populations – apparent competition (87).

VI. Selection of Repertoires. Tolerance and Autoimmunity.

In an immune system where the total number of cells is limited, lymphocyte repertoires will be shaped by the differential ability of lymphocytes to survive. Lymphocyte survival relies on cell/ ligand interactions, the availability of other "resources" and the nature and number of competing rivals.

In their continuous flight for survival lymphocytes must acquire a selective advantage over its competitors. They must adapt to the immediate environment either by modifying their survival thresholds (140) and requirements through differentiation. At different life stages lymphocytes will require different signals to survive. These factors impinge a continuous selective pressure throughout the lymphocyte life history.

Initially, T and B cell repertoires are shaped in primary lymphoid organs at early stages of T and B cell differentiation (141, 142). In the thymus, selection is determined

by the avidity of cognate receptor/ ligand interactions; by the presence of growth and differentiation factors; by the size of the selecting niche (143); by the number of competitors present at the different stages/ compartments of lymphocyte differentiation (90, 144). Immature B cell precursors are particularly sensitive to deletion and receptor-editing mechanisms by low affinity interactions (145) and the outcome of the ligand/ B cell interaction may be determined by the degree of receptor engagement (146). Selection of T and B cells continues at the periphery (147, 148). After activation lymphocytes may follow a pre-established hierarchical program and differentiate first to effector functions, then to memory, to anergy and/ or terminal differentiation (149). The duration of antigen persistence may be fundamental to trigger sequentially these stochastic processes (29, 150) and thus to determine the class of immune responses.

Lymphocyte selection and adaptation also requires co-evolution involving several different cell lines. Different subclasses of lymphocytes and antigen presenting cells represent patterns of co-evolved interactions. These patterns allow the emergence of the holistic properties of the immune system. The global properties of cellular communities by far exceed the simple properties of the individual cells. The multiple interactions between all different cells suffices to explain the immune system's development and functions.

The context of homeostasis, survival and competition excludes any manicheism as realistic to explain the global behavior of the immune system. In contrast to the original postulate of the clonal selection theory (151), lymphocyte selection through competition occurs even in absence of exogenous antigens. The idiotypic network of variable to variable region interactions is not essential (152). A closed autistic self-referential immune system (153) is impossible. The immune system's functions cannot be directed

by self/ non-self (151) or sense/ non-sense (154) discrimination or the detection of danger (155). It is likely that lymphocyte activation, rather than be determined by the ability of the cells to discriminate between one or two signals (156) will be triggered by the capacity of the cell to integrate different exogenous stimuli. Indeed, it was shown that both TCR and BCR are capable of differential signaling according to the properties of the antigen and to the environmental conditions of stimulation (63, 132).

In a complex system where a vast number of different new cell specificities are continuously generated both in the central and peripheral compartments the presence of "self-reactive" cells is unavoidable. Besides the described mechanisms of tolerance induction that work at a single cell level the global properties of the system may also contribute to avoid auto-aggression.

One of these properties is niche segregation. When a lymphocyte encounters an activating ligand in the correct niche and in the presence of plenty of resources it will respond and expand proportionally. This is the case for infectious agents which are presented to lymphocytes in the appropriate niche (in the secondary lymphoid organs) and in the presence of an excess of new resources provided by the primary inflammatory reaction. In contrast encounters with a self ligand frequently occur outside lymphoid organs, where deficient antigen presentation and limiting resources may be major factors to restrict clonal expansion and auto-aggression.

The growth of the potentially "harmful" cells will be controlled by lymphocyte competition. In these circumstances, lymphocyte diversity associated with policlonal stimulation may help preventing the emergency of large clone sizes, clonal dominance and immune-pathology. Conversely, pauciclonal repertoires will determine higher fluctuations in clonal frequencies which may increase the probability for the expansion

and fixation of clones that in absence of competition will become irreversibly dominant and induce pathology. This is supported by the inverse correlation between the world incidence of auto-immune diseases and the frequency of infectious diseases. The prevalence of auto-immune pathology is higher in developed countries with low population densities and very cold climates that disfavor germ spread. It is lower in highly populated areas of third world countries (157). In this context policlonal activation may have a therapeutic role in auto-immune disease, while immune suppression, as a side effect may reinforce the already established self-reactive clonal drift. It may be more appropriate to refer to "horror monoclonicus" rather than "horror autotoxicus": in the survival/ competition model the question is no longer how large should the repertoire be to discriminate self and non-self (158, 159), but rather what is the minimum size of the repertoire that protects and avoids auto-immune pathology.

In their flight for survival lymphocytes may be caught in a Prisoner's Dilemma game (160-162): they either cooperate, i.e. protect the host with strong immune responses, and continue to survive or they defect i.e. fail to protect or destroy the host with uncontrolled responses, and eventually die.

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