Survival of the fittest clone: Pro-apoptotic protein Noxa controls selection of lymphocytes under competitive conditions
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CHAPTER VIII:

General Discussion

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Introduction

Cells from the hematopoietic compartment, and then especially T and B lymphocytes, have to endure large amounts of physiological stress. Unlike most cells in the body, lymphocytes undergo various stages of positive and negative selection based on interactions of cell surface molecules with their environment. Unlike most cells, lymphocytes are not fixed to a specific location, but migrate continuously through the entire body, which means that they have to adapt constantly to different tissue milieus and have to interact with different adhesion proteins in their environment. Upon antigen recognition, lymphocytes have to divide tremendously, followed by differentiation and deployment of a biochemical arsenal which is potentially as dangerous to themselves as to the infected tissues at which they are targeted. Upon antigenic clearance, almost the entire pool of obsolete weaponry has to be eliminated in an orderly fashion, leaving only a small pool of hyper-responsive memory cells.

Not surprisingly, this population of hazardous, hypersensitive cells is controlled by a binary switch: survival or cell death. Only when all requirements for their persistence are met is an immune cell allowed to survive. When a single condition is not fulfilled, lymphocytes are implacably induced to undergo apoptosis. On a single cell level, this method of regulation may seem wasteful and inefficient. However, when the hematopoietic system is seen as a single organ, regulation via cell death allows a remarkably accurate fine-tuning of the behavior of this organ, expertly adapted to respond to all the stressful changes in environment described above.

This summary will elaborate on the way apoptosis is involved in mediating immunological responses on a population level. A special focus will be on the role of programmed cell death in the control of differentiated cell populations that arise upon antigen encounter and re-encounter. Primarily T and B cell biology will be addressed, but also the formation of other hematopoietic cells will be briefly highlighted.

Levels of control

Activation of mature, naïve lymphocytes is primarily controlled by interactions between their antigen receptor and the ligand to which their unique receptor is targeted. Remarkably, the affinity of this receptor is initially relatively low, especially for the T cell receptor (TCR). The Kd of a TCR for ‘its’ MHC/peptide complex is typically in the range of 1-100 µM (1), whereas that for example of an adhesion molecule for its ligand, such as VCAM for VLA4, is in the range of 10 nM (2). Activation of a T cell by TCR ligation therefore generally leads to recruitment of multiple additional adhesion and signaling molecules around the TCR and its ligand, both on the T cell and on the antigen presenting cell (3). The intercellular structure that is created is called the immunological synapse and this signaling complex contributes to the activation of a naïve lymphocyte (4).

Despite its relatively low affinity, the strength of interactions between the antigen-receptor and its ligand determines the interactions of the whole immunological synapse. A minimal binding affinity of the TCR for its MHC/peptide is required in order to induce activation (5). This threshold is influenced by the amount of cofactors, such as CD4 or CD8 for T cells and CD19 for B cells, and the avidity of antigen receptors in the immunological synapse (6,7). When the minimum affinity threshold is met, even a single MHC/peptide
complex is capable of inducing T cell responses (8). However, reaching the activation threshold does not lead to a uniform response for all activating ligands. Even when activation is established, affinity is an important determinant for the nature of the response of the lymphocyte. Low affinity ligands cause reduced signaling and lead to impaired activation, proliferation and survival (9-11). Low-affinity stimulation leads to different levels of activation of transcription factors such as NF-AT, NF-κB and AP-1 than high-affinity stimulation, thus leading to different responses (12,13).

Even when the affinity and avidity of antigen-receptors and their ligands can be accurately determined, in vivo responses can not be directly extrapolated. In fact, when a lymphocyte exclusively engages an APC via its antigen-receptor, rapid apoptotic cell death ensues, especially in B cells (14). According to the ‘three signal’ model, a naïve lymphocyte requires co-stimulation in addition to antigen-receptor stimulation in order to be allowed to survive its activation. Also, it needs a specific cytokine milieu in order to polarize its differentiation (15). For T cells, co-stimulation is primarily provided by B7 molecules, which are expressed on dendritic cells (DCs) and bind to CD28 on the activated T cell (16). B cells require binding of CD40L which is expressed on DC’s and activated helper T cells (17). Other molecules than these may also provide co-stimulation, yet their presence appears to be less indispensable for mediating immune responses (18). Also, their effects appear to be more specialized (18). Co-stimulation of lymphocytes has intracellular effects that partially overlap with those of the antigen-receptor and are partially unique. Most importantly, co-stimulation allows persistent activation signaling into the cell by enhancing activation of transcription factors such as NF-κB (19), enhancing cell cycle progression by regulation of molecules such as cyclins (20) and increasing survival by up-regulation of anti-apoptotic molecules such as Bcl-XL (21). Moreover, co-stimulation modulates expression of nutrient receptors, thus providing the requirements for the increased metabolism of activated, proliferating lymphocytes (22).

The cytokines required for activated lymphocyte differentiation and survival, the ‘third signal’, can be derived from a multitude of cell types and include many structurally and functionally distinct soluble factors, including the interleukins, interferons and TNF family members (23,24). Activated APCs are an important source of cytokines, but also helper T cells, NK cells, stromal cells, myeloid cells, infected tissues and even the activated lymphocytes themselves provide cytokines that drive differentiation (25,26). The rationale behind this multitude of differentiating signals is that it depends on the nature of infection which immunological response is required (15). Each of the cytokines has a specific function in mediating responses of activated lymphocytes. No single factor appears to be absolutely required, as many cytokines have redundant effects (27). However, when cytokine signaling is severely impaired, such as in people with deficiency for the common-γ chain, an essential structural protein shared by the receptors for IL-2, -4, -7, -9, -15 and -21, severely combined immunodeficiency ensues (28).

To summarize this section, activation of lymphocytes requires antigen engagement by the antigen-receptor, co-stimulation and the presence of a polarizing cytokine milieu. The nature of lymphocyte responses depends on antigen-receptor affinity, the kind of co-stimulation and the composition of the cytokine environment.
Competition

In addition to the three factors described in the previous section, i.e. antigen-receptor engagement, co-stimulation and cytokines, there is a fourth factor which is a major determinant for the outcome of the response of an individual, activated lymphocyte: other activated lymphocytes. The ‘three signal’ model is based on a hypothetical situation in which every cell acquires equal access to antigen-receptor stimulation, co-factors and cytokines. In vivo, however, the amount of available signals is restricted to the niche in which they reside. The central concept is that lymphocyte population size is limited by the availability of resources.

Limits of lymphocyte expansion already play an important role during homeostasis. In the absence of pathogens, the naive lymphocyte pool is maintained by a constant generation of new cells, which is balanced by apoptotic death of the cellular surplus (29). The primary cause of cell death is thought to be ‘trophic deprivation’, i.e. death of cells that have expanded beyond the sustaining capacity of the niche (24). When lymphopenia is induced experimentally, for example by lethal irradiation, the absence of cells in an intact niche allows rapid expansion of lymphocytes after bone marrow transplantation (30). The level to which these cells expand is not limited by the amount of precursors (in principle, a single hematopoietic stem cell can give rise to a complete hematopoietic compartment), but by the amount of resources that are available to support a certain population size (31). Ultimately, the same number of lymphocytes will therefore populate the host as before irradiation. Conversely, when cells are transferred into a host with a complete lymphocyte compartment, cell death of surplus cells by trophic apoptosis will rapidly lead to reestablishment of the original cell number (32). Many factors are involved in the maintenance of lymphocyte homeostasis. A primary role for the survival of naïve cells is IL-7, and mice deficient for this molecule suffer from severe lymphopenia (33).

Activation induces radical changes in requirements of lymphocytes for their persistence and survival. Of primary importance is signaling via the antigen-receptor, especially during the first hours of activation (34). In addition, the dependence of environmental factors is altered. For example activated T cells lose their dependence of IL-7 and instead require IL-2 and IL-15 for their survival (35). However, the same basic principle for the maximum population size applies; expansion of activated cells may not occur beyond the number of cells that can be supported by a given niche. When an infection is severe, more inflammatory cytokines are produced and more cells may be supported. Minor infections generate little inflammation, leading to weak lymphocyte expansion.

The fact that apoptosis plays an important role in limiting population size is demonstrated in mice lacking apoptosis regulators. Bim is important in mediating cell death as a result of IL-7 and IL-15 deprivation (36,37). Bim-deficient animals rapidly accumulate naïve T and B cells under conditions of homeostasis, indicating that trophic cell death as a result of limiting IL-7 levels is reduced in these mice (38). During acute infection, Bim−/− mice generate more antigen-specific cells, suggesting reduced dependence of IL-15, which allows enhanced expansion (39). In addition to Bim, various other apoptotic proteins are thought to play a role in mediating lymphocyte population size during homeostasis and activation. Mcl-1, a pro-survival member of the Bcl-2 family of proteins, is strongly upregulated during lymphocyte activation and its levels respond heavily to the amount of available IL-2 (Chapter
4 and (40)). Puma, a pro-apoptotic family member of Bim, is also induced upon cytokine deprivation and mice deficient for this protein have enlarged T cell responses during infection (41). Previously, our group showed that, in addition to cytokines, also nutrient availability is an important determinant for mediating lymphocyte responses. Noxa, a pro-apoptotic protein that is strongly induced in activated lymphocytes, mediates cell death in response to glucose deprivation (42). It was therefore proposed that, when cells compete for nutrients during an immune response, Noxa may mediate lymphocyte expansion (43).

Despite their common goal, i.e. clearance of the pathogen, lymphocytes are in direct competition with each other for the limited amount of antigen, co-stimulation, cytokines and nutrients, for convenience called ‘immunological space’ here. If all lymphocytes would be identical, no cell would have an intrinsic advantage over the other and trophic survival would be determined by location. Cells that acquired access to a certain niche would have a survival advantage over cells that could not. It has been proposed that such is the case for long-lived plasmacells (44). These cells have no antigen-receptors on their surface and all plasmacells are therefore in principle equal when competing for a niche. Survival of these cells is primarily ensured by their ability to reach the supporting niche in the bone marrow (44).

Lymphocytes, however, are all different by default, as each cell carries a basically unique antigen-receptor. Since, as discussed in the previous section, the antigen-receptor gives the primary signal for lymphocyte activation and since binding strength of this signaling complex has a major influence on the activation status of the cell, antigen-affinity is therefore a likely determinant for competitive performance of activated cells. Indications that competition plays a major role in mediating immune responses have come from experiments using cells from transgenic mice. When B cell receptor (BCR) transgenic B cells of high or low affinity are transferred in separate mice, no major differences are observed in B cell expansion or in the amount of antibodies produced upon immunization. However, when these cells are co-transferred into a host, high-affinity cells have a clear survival advantage (45,46). Already before the germinal center reaction, high-affinity cells overgrow their low-affinity competitors. Also in T cell independent responses this effect was observed (46). In the germinal center, where competition is exceptionally high, this competitive advantage is more pronounced and leads to increased B cell numbers and amounts of antibodies produced by high-affinity cells. It was also shown that, at least in the germinal center, apoptosis plays an important role during this competition, as low-affinity cells display higher levels of apoptosis than high-affinity cells (47). For T cells, this competitive survival effect has been less thoroughly investigated. What is clear is that TCR affinity has an influence on T cell survival in vitro (11).

In summary, activated lymphocyte performance is controlled by the amount of resources that make up the immunological space. In a situation of rapidly expanding lymphocytes, such as is the case in the lymph node during a primary infection, the availability of resources is not just limited by the production of these factors by the niche. Also the capacity of activated lymphocytes to obtain these resources during competing with each other plays an important role. Since primarily the antigen-receptor determines the activation status of the lymphocyte, antigen-affinity is therefore an important factor for the competitive performance of activated lymphocytes. We argue that apoptosis of low-affinity cells, or
survival of the fittest’ (i.e. high-affinity) lymphocytes, plays an important role in this competitive environment.

**The role of Noxa in competition and selection**

In chapter 4 we describe a molecular basis for this competitive model in T cells. T cell activation leads to induction of Mcl-1 and its antagonist Noxa. Protein levels of Mcl-1 are maintained only in the presence of autocrine IL-2 signaling, which is directly dependent on antigen-affinity. Low-affinity TCR ligation leads to low levels of CD25 (the IL-2 receptor subunit α) expression and rapid loss of Mcl-1. High-affinity triggering leads to high-levels of CD25 and maintenance of Mcl-1 protein. Loss of the Mcl-1 antagonist Noxa thus leads to a competitive advantage of these T cells over wild type lymphocytes, especially under conditions of low-affinity TCR stimulation. *In vivo* this results in persistent survival of low-affinity clones and thus increased clonal diversity of the antigen-specific effector cell pool in Noxa-deficient animals. Since the overall effector pool size remains the same in these mice, they therefore have reduced amounts of high-affinity clones upon viral infection. The physiological consequence of this effect is that viral clearance is delayed in Noxa−/− mice due to reduced effectiveness of the effector T cell pool. Thus, apoptosis of individual cells determines the behavior of the effector T cell population as a whole (*Figure 1*).

In chapter 6, we applied the same model to B cells and observed that a similar mechanism controls selection of high-affinity B lymphocytes. Activation of B cells leads to induction of Mcl-1 and Noxa. Deficiency for Noxa results in reduced apoptosis of B cells survival of low-affinity clones. Persistent survival of low affinity cells decreased the available immunological space for high-affinity cells, thus leading to reduced outgrowth of high-affinity clones. Again, this indicated that apoptosis of individual cells affected the affinity and behavior of the entire effector pool.

Interestingly, when studying memory responses, we found major differences for the role of Noxa between T cells and B cells. Boosting of Noxa deficient animals in order to look after activation *in vitro* and *in vivo*, and to enlarged germinal centers. When directly competing *in vivo*, Noxa deficient cells had a survival advantage over wild type cells. Phenotypic and functional analysis of these cells and of the antibodies they produce revealed that they were of reduced affinity for the antigen with which the mice were immunized or infected. On a molecular level we could corroborate this with increased clonal diversity, indicating that the reduced affinity of the effector B cell population was the result of increased

*at memory B cell responses resulted in impaired antibody production and a further increase in antibody-affinity differences between Wild type and Noxa+/− mice. This occurred despite the presence of increased memory cell numbers in animals lacking the Noxa gene (Chapter 6). When studying memory T cell responses, on the other hand, we found that initial affinity differences of primary effector cells and also memory cells between wild type and Noxa+/− mice were progressively lost upon re-encounter of antigen (chapter 5). Rather, Noxa appeared to mediate the effector T cell population size as Noxa deficient mice had enlarged recall responses upon antigen re-encounter. During chronic viral infection of Noxa+/− animals, this led to effector T cell accumulation and resulted in severe organ pathology and*
Figure 1. Model for the role of Bcl-2 molecules during antigen-driven effector T cell selection.

(a) Regulation of Bcl-2 molecules on a single cell level. (i) In inactivate, naïve T cells, the anti-apoptotic molecules balance the function of pro-apoptotic BH3-only molecules. Upon activation this equilibrium is shifted, with a rapid stabilisation of Mcl-1 and an increase of Bcl-XL and A1 proteins. After several days Noxa levels increase, resulting in competition with Bim for binding places on Mcl-1. Noxa-bound Mcl-1 is degraded by the proteasome, resulting in reduced Mcl-1 levels. (ii) When the antigen-affinity of the TCR is high, Mcl-1 levels are persistently stabilised via cytokine signalling, and continue to outnumber the amount of pro-apoptotic BH3-only molecules, resulting in cell survival. (iii) When the antigen-affinity of the TCR is low, Mcl-1 levels are reduced, resulting in free Bim, which can subsequently antagonise other pro-survival molecules, such as Bcl-XL and A1. When the pool of Bcl-2 like proteins is saturated with BH3-only molecules eventually Bax and/or Bak are activated, resulting in cell death. Here, the situation according to the direct activation model (Willis and Adams, 2005) is depicted. Indirect activation will, however, lead to the same model concerning T cell selection. (b) Regulation of antigen-driven selection by apoptosis and proliferation on a population level. Upon antigen encounter by the naïve T cell pool, the majority of T cell clones will not bind and remain inactive (non-responders), but per epitope a small number of T cell clones surpassing the affinity threshold will be activated and start proliferating. During proliferation, competition will occur between these clones for antigen, cytokines and nutrients, with an advantage for high-affinity clones, as outlined in a. These cells will most efficiently up-regulate receptors for survival and proliferation signals and this will lead to increased McI-1 stabilization. Thus these cells are allowed to overcome the survival threshold set by the balance between pro- and anti-apoptotic molecules and continue to proliferate. Low affinity-clones are not able to reach this threshold and will perish by apoptosis.

premature death in a transgenic model of persistent T cell activation. This is in stark contrast with primary responses, where the effector T cell population size is not different between wild type and Noxa<sup>-/-</sup> mice (chapter 4).
We hypothesize that limits of immunological space underlie all these observations and that Noxa-mediated apoptosis plays a central role in controlling trophic cell death. Reasons for the observed differences are most likely caused by differences in requirements for the immunological space between naïve and memory cells and B and T cells. In addition, the competitive environment differs drastically for these different cell types. Naïve T and B cells, for example, are primed in the lymph node and this is also the place where they rapidly expand. Interclonal competition therefore plays an important role in limiting expansion of sub-optimally activated cells. Murine memory B cells are poorly defined. However, upon boosting, enhanced B cell responses are observed in the lymphoid organs of both humans and mice, resulting in the generation of large germinal centers there (48,49). Interclonal competition will thus also play an important role in secondary B cell expansion. Since Noxa mediates survival during interclonal competition, we predict that, as during priming, memory B cell responses are impaired in these animals due to increased survival of subdominant clones. These cells limit immunological space for high-affinity cells and therefore inhibit their expansion.

Even though memory T cells are also found in the lymph nodes, a large fraction of these cells resides in non-lymphoid tissues. Especially for respiratory viruses, there are indications that antigen-specific cells preferably reside in the organs where the primary infection originated (50). Re-encounter of pathogens therefore leads to T cell expansion at multiple sites, also outside of the lymph nodes. Since the density of different expanding clones is much smaller in these organs than in the lymph node, interclonal competition will therefore probably be of reduced importance. We think that this is the reason why differences in antigen affinity between Wild type and Noxa deficient effector T cells were only observed upon primary expansion. However, also in inflamed tissues the immunological space is ultimately limiting. As previously mentioned, effector cell expansion is controlled by the presence of inflammatory cytokines such as IL-15, which are highly produced in inflamed tissues (51). Since Noxa was previously shown to mediate cell death in response to IL-15 deprivation in NK cells (37) and since Noxa is highly expressed in activated T cells, we propose that increased effector T cell expansion upon secondary infection is the result of reduced dependence of IL-15 in Noxa−/− cells.

In chapter 2 we attempted to find the link between competition (i.e. limitations of the immunological space) and apoptosis induction. Our focus in this chapter was on limitations of nutrient availability, rather than cytokine deprivation. Noxa-mediated apoptosis was shown to be accompanied by proteasomal degradation of Mcl-1 during general metabolic stress. Using a model of leukemic T cells or erythroleukemic myeloid cells we found that several major stress responses, including AMPK, GSK3β and MAP kinase signaling did not provide the link between metabolic stress and Noxa-mediated apoptosis induction or Mcl-1 degradation. Also, we found no major role for autophagy and we observed that the formation of reactive oxygen species was not an upstream event for inducing programmed cell death in this setting. We did show that general metabolic stress does not exclusively require Noxa, but is also influenced by Bcl-2 family members such as Bim and Bcl-2. Thus, even though we could exclude several of the ‘usual suspects’ of apoptotic signaling in response to general nutrient-deprivation driven stress, the actual link between metabolic sensing and apoptosis in primary and leukemic lymphocytes is still unclear.
In summary, in chapters 2, 4, 5 and 6 we describe how Noxa-mediated apoptosis functions as a control mechanism for the selective outgrowth of high affinity cells when activated B or T cell clones compete with each other for limited immunological space in the lymph node. During memory T cell expansion upon antigen re-encounter, Noxa controls the maximum population size rather than clonal composition of the effector cell pool. How Noxa-mediated apoptosis is triggered in response to limiting resources during lymphocyte expansion still needs to be investigated. What is clear is that both IL-2 deprivation and general metabolic stress both play an important role.

Linking apoptosis sensitivity to immunological space by co-stimulation

As already mentioned in the previous sections, survival and proliferation of activated naïve lymphocytes is controlled by the availability of antigen, co-stimulation and cytokines in the lymph node. The limitations of the immunological space are, however, not only restricted by available resources, but also by the status of the cells present in that niche. In other words, availability does not only depend on how much antigen, cytokines and co-stimulatory proteins are present in a niche, but also on how much of these triggers a specific cell in this niche minimally needs to function. For example, when a cell is co-stimulated by B7 proteins via CD28 it up- and down-regulates other molecules than a cell which is co-stimulated by CD70 via CD27 (our observations and (52)). The need for resources of these two cells differs and thus the limit of their expansion when these cells are placed in the same niche. Experimentally this status can be influenced in various ways, for example pharmacologically (53) or by genetic removal of apoptotic molecules. In Bim^-/- mice, the ‘status’ of lymphocytes is altered, leading to a higher tolerance of low homeostatic cytokine concentrations and thus to a different limit of expansion (38-40).

Combined, in chapters 4 and 7 we show how co-stimulation has a divergent influence on these intrinsic requirements. A major difference between CD28 and CD27 co-stimulation was downregulation of pro-apoptotic molecule Bim after CD27 stimulation. Triggering of CD27 during stimulation of the TCR thus leads to a different balance of pro- and anti-apoptotic molecules, changing their sensitivity to apoptotic stimuli and altering their minimal requirements for available resources. Interestingly, apart from influencing the sensitivity of cells to limitations of available resources, CD27 triggering also mediated the responsiveness of cells to inhibitory proteins. CD27 co-stimulation lead to up-regulation of FASL and engagement of the FAS (CD95) mediated pathway of apoptosis. This suggests that CD27 co-stimulation indeed positively influences survival of activated T cells by manipulation of the balance between pro- and anti-apoptotic proteins, but only under conditions where FAS engagement is prevented. Our findings illustrate that T cell expansion is not only passively restricted by the limited availability of resources and their effect on the internal status of the cell, but also actively via inhibitory signals.

Competition among equals

We mentioned in a previous section that when all cells in a given niche are identical, no cell has an intrinsic advantage over the other and trophic survival is determined by location. In chapter 3 we investigated this situation for erythroid precursors. These cells are in
principle all the same and their requirements are determined by their differentiation status and the niche in which they reside. Most erythropoiesis occurs in the bone marrow, where resources for erythroid development are abundant (54). Under homeostatic conditions, in mice only a small amount of extra-medullary erythropoiesis takes place in the spleen, where primarily the levels of the cytokine erythropoietin (epo) are limiting (55). Trophic cell death is also an important control mechanism of the extra-medullary erythoblast pool size, as expansion beyond the maximum number that can be sustained by the splenic erythoblast niche leads to induction of apoptosis (56).

In chapter 3 we discovered that Noxa is induced during erythroid differentiation of both human and murine stem cells and that this protein mediates apoptosis of these cells in response to cytokine deprivation. Noxa-deficient erythroblasts therefore had reduced sensitivity to cytokine deprivation, leading to reduced trophic apoptosis of these cells under homeostatic conditions and increased numbers of extra-medullary red blood cell precursors in Noxa−/− mice. When the extra-medullary niche was increased by induction of anemia and increase of epo levels in the serum, trophic cell death was reduced and similar (increased) levels of erythroid precursors were found in Noxa−/− and wild type spleens.

These data show that also within cell populations that seem outwardly identical, a selection takes place that favors survival of some cells and death of others. Possibly this selection is purely the result of a process controlled by chance, allowing some cells to reach the required resources based on no competitive advantage at all. Alternatively, there may still be competition taking place based on cell intrinsic features, for example as a result of subtle changes in Bcl-2 family members and apoptosis sensitivity as differentiation along the erythroid lineage progresses. Future research must reveal which of these options is true or that both processes occur simultaneously. What is clear is that also in erythropoiesis, apoptosis of individual cells functions as a mechanism to control the behavior of the red blood cell pool as a whole.

Future prospects and concluding remarks

Apart from adding to our general scientific understanding of immune responses, we hope that our results will contribute to the advancement of clinically relevant research, especially on the field of vaccine design. In this last section we want to briefly address this topic, and give our view how we think insights in biology of competitive lymphocytes and the immunological space in which they reside may help us in the clinic.

Currently, vaccines are primarily focused on generating a large immune response. Sometimes, an adjuvant is added to the injected cocktail in order to activate as many factors involved in generating lasting immunity. However, as previously mentioned, each infection activates a highly specialized subset of cells in order to steer the immune response in the most efficient way to fight that specific pathogen (15). Therefore, to generate successful therapies to prevent infection with pathogens, such as HIV, which still evade immunity generated by current vaccines, a more specialized immune response is required.

One strategy to generate a more pathogen-specific response may be by influencing the nature of the immunological space, for example by adding cytokines in the vaccine cocktail. Cytokine-enriched vaccines could potentially polarize the differentiation of immune cells, thus leading to a more efficient response. Alternatively, we may attempt to change the ‘status’
of the immune cell. By including drugs such as ABT-737, which mimics pro-apoptotic Bcl-2 family members, vaccines may alter the intracellular sensitivity of cells to the immunological space (57). As we have shown that this intracellular status is of great importance for the affinity of the effector cell pool (chapters 4 and 6), such compounds may be of benefit for narrowing the clonal response, thus increasing its specificity. Conversely, we may try to prevent apoptosis in order to generate a more broad neutralizing response. However, manipulation of the balance between Bcl-2 proteins influences the intracellular apoptotic status of all cells in the body and is therefore likely to have serious side effects for inclusion in a vaccine. A similar argument holds true for cytokines, which can induce broad inflammatory responses and severe tissue damage (58). These therapies may therefore not be applicable for all situations.

Co-stimulatory molecules, on the other hand, are much more restricted in their effects and are highly specific for T and B cells. Manipulation of the status of activated lymphocytes via these natural receptors is therefore a promising target. Currently, many antibody-based therapies are under development (59,60) and future research must reveal whether we can use these not only to enlarge immune responses, but also to direct them towards the preferred differentiation pathways. However, lessons from the past have learned that even specific therapies may have severe adverse effects (61). Also, our findings in chapter 7 concerning the bifurcate role of CD27 in immune responses indicate that the same therapy may have positive effects during acute infection, but adverse effects for chronic responses.

Therefore, we may want to adopt different strategies dependent on the infection we want to prevent. In the development of an HIV vaccine, a broad neutralizing response seems more efficient. A vaccination against this pathogen will benefit from Noxa inhibition and/or stimulation of Mcl-1 in T cells, for example via IL-2 therapy. When a narrow response is preferable, such as when a tumor vaccine is developed, antigen-specific lymphocyte survival must be restricted to only the most specific cells. Inhibition of co-stimulation, for example with antagonizing CD27 antibodies, sustains Bim expression and reduces induction of Bcl-XL. This allows survival of only those cells that maintain viability via high-affinity driven Mcl-1 stabilization and thus narrows the immune response. Future research must reveal whether such therapies are possible, but in our opinion they do pose a promising target.

In conclusion, the nature of immunological responses is the result of a delicate balance between cues from environmental limits, cell-intrinsic factors and randomly occurring stimuli from invading pathogens. In this thesis we have tried to gain more insight in the role that apoptosis plays in keeping this balance. We investigated how the behavior of entire cell populations of the hematopoietic compartment was influenced by the death of individual cells via Noxa mediated apoptosis. We found that what applies to species in nature, also applies to differentiated pools of cells within an organism. Many times, death of individual cells makes the remaining group stronger, weeding out ineffective clones to allow the unhindered expansion of high-affinity killers of infectious agents. Apoptosis of cells that expand beyond the amount that can be sustained is necessary, as excessive proliferation may lead to depletion of nutrients or accumulation of cytotoxic cells that ultimately destroy the own body.
And finally we find that death of the individual cell is not an end at all, but part of an ongoing balance between expansion and contraction, of recycling and renewal, allowing the hematopoietic compartment to function as one single organ, continuously.
Reference List


