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THE DEVELOPMENT OF THE SPECIFICITY REPERTOIRE OF THE IMMUNE SYSTEM: REVIEW OF THE INTERNAL DISCUSSION

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INTRODUCTION

Before we can attempt to understand the complex interactions between the immune system and the host's microflora, we have to understand the dynamics of the contacts between both from the first day after birth e.g. the ontogeny of the immune system. In other words we need information about the immune system before and after exposure to external antigens.

During a one-and-a-half day internal discussion between the speakers of the 6th Old Herborn University Seminar some elements of the ontogeny of the immune system have been discussed. This report focuses on 5 different topics: Early B cell development, B cell repertoire selection during development, function and development of CD5 B cells, T cell repertoire selection during development, and finally tolerance and self assertion. Discussion of each topic was introduced and chaired by one or the speakers invited (names between brackets). This report reflects the important issues raised.

EARLY B CELL DEVELOPMENT
(M.D. Cooper)

Ontogeny of the B cell system

Humans have a very long gestational period of 40 weeks. The first B cells are seen around 7 weeks of gestation. B cells of all isotypes, as detected by conventional antisera, reach adult frequencies around 10 to 12 weeks of foetal life. First IgM plasma cells are seen around week 12. IgG plasma cells appear somewhat later, around week 20, and finally IgA plasma cells appear at week 32. Most plasma cells are located in spleen and bone marrow.

Maternal IgG peaks around birth (week 40). These antibodies gradually disappear and are not detectable one year later. Serum IgM appears late in gestation (before birth) and reaches adult levels at one year of age. Foetal serum IgG and IgA appear later, around birth. IgG reaches adult levels at 5 to 6 years of age, whereas serum IgA reaches a mature level at puberty.

Factors involved in the functional delay of B cells

Development in foetus and new-borns

Although B cells of all isotypes are present early in the development of the foetus, serum immunoglobulins appear

very late. Apparently a delay in the (terminal) differentiation of the B cell population is present. This has clinical significance e.g. the immune response against pneumococcal antigen is severely impaired during the first 1 to 2 years after birth.

A major question is how to explain this apparent delay. Possible explanations may include:
1. the inability of the B cells to respond,
2. T cell regulatory mechanisms, and/or
3. environmental factors.

Ad 1:

Neonatal/foetal B cells can be an immature population or different population of cells which cannot differentiate. Support for this is that non specific stimulation *in vitro* with mitogens or EBV transformation of newborn B cells (i.e. 20-30% of the lymphocytes in circulation) results in secretion of IgM, whereas almost no IgG production and even no IgA production is found (although initially 5% of the B cells are surface IgA positive). Other data have shown that marginal zone B cells in the spleen are not fully developed (CD2l down regulated) until 2-3 years after birth. During foetal life the B cell repertoire is limited/restricted: between 7 and 8 weeks of gestation exclusively V_H5/6 is present. Later (week 8) B cells also express V_H3 until close to birth when other V_H genes are expressed as well. It is not known whether these preferential rearrangements are due to selection mechanisms. N-sequence additions start very early during gestation and numbers of insertions gradually increase during foetal life.

Ad 2:

T cells in the new-born might be different from adult T cells. Indeed, virtually all Th cells are Th2 cells. Th1 cells appear to lack. This may result in differences in lymphokine profiles in newborns, which in turn may explain the relative absence of isotypes other than IgM. Furthermore, also CD45 R0 cells appear to be absent and 45% of cord blood T cells are CD45 RA positive. The vast majority of these RA^+ cells express the CD38 marker, which is associated with activation. CD45 RA cells in adults are CD38 negative. This particular subpopulation of CD48 RA and CD38 double positive cells is believed to have a specific suppressive effect.

Ad 3:

The different microenvironment of new-borns can also contribute to the delay in antibody production of various isotypes. The intestinal microflora in the neonate differs substantially from the adult. It is also possible that antigen presenting cells are functionally absent, e.g. it is known that mature FDC are absent in rat spleen until 2 weeks after birth (*Kroese*). However, transfer of adult B and T cells to neonatal mice results in normal adult level responses upon antigenic stimulation (*Coutinho*).

Development after bone marrow transplantation

After bone marrow transplantation (BMT) to supply haemopoietic stem cells (haematological malignancies), erythrocytes and myeloid cells repopulate quickly. However, it takes much longer to functionally regenerate the immune system. In this respect, the development of T and B cells after BMT is similar to the neonatal development of the immune system. BMT patients cannot generate adequate antibody responses of all isotypes upon antigenic stimulation one year after transfer. The 'cleaner' the preparation of haemopoietic stem cells (BM-graft) the longer it takes to regenerate the immune system. This is likely due to absence of outgrowth of mature (contaminating) lymphocytes.

B CELL REPERTOIRE SELECTION DURING DEVELOPMENT

(K. Rajewsky)

Relatively little is known of the establishment of the B cell repertoire. By contrast, for T cells there is good evidence for both negative (active deletion) and positive selection (active survival). Negative selection, for example, is seen as physical deletion of thymocytes after interaction with superantigens in the thymus. Positive selection occurs after interaction of T cell receptors on thymocytes with environmental (MHC class II) antigens or peripheral T cells that interact with classical exogenous antigens. Two levels of B cell selection can be distinguished:

1. Selection in the bone marrow (BM),
2. Selection during the generation in the germinal centres.

Here we will consider positive and negative selection at these two levels.

Selection of repertoire from progenitor B cell to mature B cell

Studies in immunoglobulin transgenic mice (e.g. *Nemazee*, *Goodnow*) have shown that negative selection can occur among BM B cells. Cells are deleted in the BM as soon as they express surface immunoglobulin specific for endogenous antigens. The mechanism for this cell death is not known and there is no evidence yet that this occurs by apoptosis. Possibly most important in this process may be the microenvironment of the BM.

Indications for positive selection of B cells come from experiments comparing the V_H gene repertoire of pre-B cells and peripheral IgM^+ IgD^+ B cells. Data show that in the stable pool of relatively long lived cells, the different numbers of the J558 family V_H genes are present at not random frequency. This selection is likely due to antigens as in germfree (GF) B6 mice the J558 V_H gene repertoire of mature splenic B cells is similar to that of pre-B cells. However, as mentioned by *Rajewsky*, one should keep in mind that GF B6 mice are only derived by caesarean section of conventional B6 mice after which the neonates are fostered by GF Balb/c parents. Another ligand that may play a role in selection of B cells is antibody itself. This was shown by experiments of *Kearney*, who treated mice from birth with anti-Id antibodies, which resulted in a significant chance of the repertoire.

Best evidence for positive selection of B cells is seen at the level of $CD5^+$ B cells (see discussion *Kearney*) as reflected by strong enrichment in V_H11 and V_H12 genes and specificities for autoantigens and antigens expressed on microorganisms.

Generation of high affinity antibodies in germinal centres

Germinal centres are clusters of B blastoid cells that develop upon antigenic stimulation in the centre of follicles. Within the germinal centre B memory cells are being formed. During this proliferation process B cells expand in an oligoclonal fashion while they undergo isotype switching and somatic mutations. B cells expressing immunoglobulins with high affinity for the antigen that induces this germinal centre reaction are rescued from cell death by positive selection. B cells with low or absent affinity are not selected and will die by apoptosis. Molecules involved are e.g. CD40 and BCL-2. In mice the most differentiated B cells have the highest level of BCL-2 compared to pre-B cells in the bone marrow that have >100 fold lower levels (*Coutinho*).

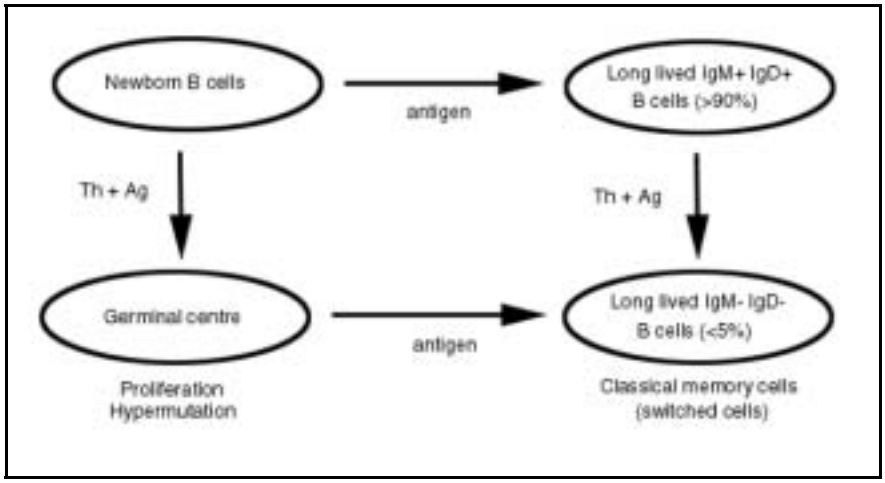

Figure 1: Hypothetical scheme of generation of peripheral B cell pool through cellular selection.

There is weak evidence for negative selection in germinal centres that might be needed in case the somatic mutations lead to autoreactivity. Signals involved in these selection phenomena might be well studied in the near future using the recently developed "*in vitro* germinal centres method".

Positive selection has also been clearly demonstrated for memory cells. Recently *Schittek* and *Rajewsky* analysed the J558 V_H gene usage of M^- D^- (memory) B cells of not deliberately immunised animals. Excitingly they show that same gene selection takes place among memory B cells as in M^+ D^+ mature B cells. Members of the J558 family used are similar to those from the pool of these M^+ D^+ B cells (see above). The only difference is that 80% of these V_H genes have somatic mutations.

Hypothetical scheme of B cell selection

During the generation of newborn B cells, cells may die if there is no good pairing of a rearranged heavy chain with a light chain. It is unknown what percentage of these cells finally produces a functional antibody and it is questionable whether this is a negative or positive selection process. It is also not known whether specificity selection occurs at the pre B cell level. However, it is known that the λ 5 light chain in combination with a μ heavy chain is expressed on pre B cells. According to one hypothesis, the role of expression of this complex is to test out the productive rearrangement of the μ heavy chain while it is not involved in a selection process. There is some evidence against negative selection at this level. Injection of anti λ 5 antibodies does not cause shifts in the repertoire. However, this might be explained by the presence of weak signal transduction mechanisms.

Data from *Holmberg* suggest that positive selection of D-proximal V_H-genes occur in perinatal life while the same genes are negatively selected in the adult BM. Another question is where the selection of newborn B cells takes place: In the BM or in the spleen or both. This question might be well addressed in rats but not in mice because only in the former species newborn B cells but not the IgM^+ IgD^+ B cells express Thy-1 allowing isolation of these cells for analysis (*Kroese*).

The generation of plasma cells is also

a selective process. Plasma cells producing isotypes other than IgM are derived from switched cells, an event that occurs in the germinal centre (GC). However, this does not imply that all switched cells must be derived from GCs. Indeed IgG and IgA producing cells are found in nude animals and antigen-free animals which lack GCs. Plasma cells in these animals, however, appear to make unmutated antibodies. In antigenfree mice the number of IgG and IgA plasma cells is 100 fold lower compared to conventional animals and their specificity repertoire resembles that of IgM plasma cells (*Bos*).

FUNCTION AND DEVELOPMENT OF B-1 CELLS (CD5 B CELLS)

(J.F. Kearney)

Lineage origin of CD5 B cells

There is still controversy about the lineage origin of CD5 expressing B cells. These B cells are now called B-l cells (previously called CD5 B cells or Ly-l B cells) and are distinct from conventional B cells in anatomical location, function (including repertoire), phenotype, and development. B-1 cells are enriched in the peritoneal and pleural cavities and produce multireactive antibodies directed to autoantigens and microorganism related antigens. The major point of discussion is whether CD5 expression on B cells defines a distinct B cell lineage with their own progenitor cells or that CD5 expression is the result of a distinct B cell activation pathway (thus, separate progenitors versus separate environments).

Transfer and transplantation studies by several groups have shown that B-1 cells are derived from progenitor cells found in foetal liver (*Herzenberg, Hardy, Kantor*) and exclusively from progenitor cells located in foetal omentum (*Kearney*), and paraaortic splanchnopleura from early (8.5-9 days) embryos (*Marcos*). Progenitor activity of adult bone marrow for B-1 cells is severely reduced (but not absent; in particular sister $CD5^-$ B-lb cells) and in the adult mouse B-1 cells maintain themselves by self-replenishment, although there are findings suggesting a pool of B-1 precursors in the adult (*Marcos*). B cells that belong to the conventional lineage are produced throughout animal life from progenitor cells located in the bone marrow. In the foetus, conventional B cells also derive from the foetal liver.

Wortis et al. speculate that B cells are not pre-committed to become B-1 cells. They claim that there are two B cell lineages, one foetal B cell lineage and one adult B cell lineage, which both can give rise to CD5 expressing B cells, after activation by certain antigens. One difference between the foetal and bone marrow lineage would be the number of N terminal sequences, inserted during the V_H gene rearrangement using the enzyme TdT. In lymphoid cells from foetal liver, TdT is absent while it is clearly present in bone marrow pro-B cells. Concomitantly, B cells derived from foetal liver almost lack N-terminal sequences. According to *Wortis*, activation of B cells from both lineages lead either to CD5 expressing B cells or B cells that have low levels of J11d ("classical memory cells"). Activation of B cells by crosslinking their sIg (e.g. by carbohydrates or anti-Ig), is thought to result in CD5 expressing B cells whereas activation of B cells in a T cell dependent fashion by non-crosslinking antigens results in J11d low cells (memory cells).

The CD5 B cell repertoire

Antibodies secreted by B-1 cells have a distinct and selected specificity repertoire. In general they produce multireactive antibodies which are enriched for auroreactivity and for epitopes expressed on microorganisms (like DEX). Antibodies produced by B-1 cells are strongly connected to each other and thus play an important role in the maintenance of the idiotypic network. At the DNA level they have a distinct V_H gene repertoire and exclusively use V_H11 and V_H12 genes and their V_H genes are usually not mutated. By these properties B-1 cells are believed to play a role in the first line of defence of the animals and to carry a kind of evolutionary memory to environmental antigens. Therefore they do not need high affinity antibodies encoded by mutated V_H genes. It is not known yet whether B-1 cells can participate in the germinal centre reaction, i.e. the sites where the somatic mutations are introduced.

The role of CD5 B cells in the mucosal immune system

Most plasma cells in the animal are IgA secreting cells, which are located in the gut wall. B-1 cells likely also contribute significantly to these IgA plasma cells as shown in transfer experiments (*Kroese*). In these studies peritoneal B cells are transferred to irradiated recipients, reconstituted with IgH-C congenic bone marrow. In these so-called lineage chimeras, only B-1 cells develop from the donor peritoneal cells and in the gut lamina propria many IgA plasma cells (up to 50%) have the allotype of the peritoneal cell donor. Similarly, SCID mice engrafted with foetal omentum, which results in development of B-l cells (but not conventional B cells), also produce IgA cells in the gut (*Kearney*). These type of experiments strongly indicate that IgA plasma cells can be derived from donor B-1 cells, in addition to conventional B cells that differentiate through Peyers patches. The relative contribution of these two different lineages in the production of gut IgA plasma cells in normal, untreated animals is not known. Furthermore whether these two IgA plasma cell populations have different repertoires and functions remains to be answered. In this context, monoclonal IgA antibodies (of unknown lineage origin) are the most polyreactive antibodies, even more than IgM antibodies (*Coutinho*).

T CELL DEVELOPMENT

(H.R. MacDonald)

Generation of T cells in the light of B cell development

Allelic exclusion of the T cell receptor

There is substantial evidence that the vast majority of B cells in normal animals express only one Ig heavy chain gene. This phenomenon is called allelic exclusion. However, data from transgenic mice suggest that endogenous genes may be expressed together with the rearranged m transgene, although controversy exists about the interpretation of these findings. Furthermore, for light chains 5-10% of hybridomas made from neonatal spleens (but not from adult spleens) simultaneously produce κ and λ light chains as demonstrated both at the protein and mRNA level (*Kearney*). These findings show that allelic exclusion for Ig light chains may not be complete in normal animals.

Recent studies with β gene transgenic mice show that in T cells allelic exclusion may also occur for TCR genes. In these mice endogenous β chain expression is inhibited by the transgenic β gene. No such allelic exclusion is found for α chains and approximately 30% of

murine T cells potentially produce two productive a chains. This has been demonstrated at the mRNA level but not yet at the protein level. Thus allelic exclusion does not seem to be absolute at the TCR level.

Regulation of rearrangement of the T cell receptor

Recombinase activity in B and T cells is associated with RAG1 expression. Although RAG1 is present during early B cell development, it is not known yet whether different levels of RAG1 can be found in different steps of B cell differentiation. Two waves of RAG1 expression are found during development of T cells in the mouse. Approximately 1% of murine thymocyte T cells has intermediate levels of RAG1 mRNA levels. These cells that express the IL2 receptor, are an early phase in T cell development and are associated with β and γ rearrangements. Later during development when the cells express CD4 and CD8, high levels of RAG1 mRNA are seen. This corresponds with rearrangement of the TCR α locus. At this phase of T cell development, further differentiation of T cells can be regulated by Ig ligating the TCR α chain, which leads to reversible staggering in development. It is not known whether there is a surface molecule expressed in conjunction with surface expression of the β chain on thymocytes early during development which parallels the expression of the λ 5 gene on pre B cells in the mouse.

T cell development

γδ T cells

With respect to the T cell receptor there are two distinct T cell populations. Two percent of the peripheral T cells express γδ TCR whereas all others express αβ. These T cells are not MHC restricted and therefore they may be generated at extrathymic locations. Indeed nude mice have γδ T cells.

Whether the two T cell populations are distinct lineages is not fully established. The low percentage of γδ T cells, present in a 1 to 50 ratio (with respect to αβ T cells), can be fully explained by the difference in the size of the coding genes (stochastic explanation). Knockout experiments show that α TCR chain knockout mice still express δ chains and vice versa δ knockout mice express α chains. Although α chains rearrange in the absence of δ chains (and vice versa), this does not need to imply that there is segregation of αβ and γδ T cells at the progenitor level.

Models for development of CD4 and CD8 T cells

An intriguing question is what determines whether a mature T cell becomes a CD4 or CD8 positive cell. At present there are two different models to explain this differential differentiation of T cells. First, according to the "instructional model" by *Von Boehmer* and others, all immature $CD4^+$ and $CD8^+$ T cells (cortical thymocytes) potentially have the capacity to differentiate either to single $CD4^+$ or $CD8^+$ cells. This final differentiation step is thought to be determined by interaction between TCR and ligand, i.e. MHC class I and II.

Secondly, in the "committed model" by *MacDonald*, T cells are already committed to become either single $CD4^+$ or $CD8^+$ cells at the $CD4^+CD8^+$ or even earlier at the $CD4^-CD8^-$ stage. The $CD4^+CD8^+$ cells only need the appropriate ligand to survive and become single $CD4^+$ or $CD8^+$ cells (experimental data by *MacDonald*).

According to which of the two models T cells differentiate is currently unknown. However, in both models T cells are positively selected upon interaction with MHC Class I or II antigens. Although most T cells are thus selected for interaction with class I or II anti-

gens, in normal mice a low percentage of the T cells appears not to be restricted heavily.

B cells in the thymus

B cells are always present in low numbers in the thymus of humans, rats and mice. Recent data indicate that thymic B cells may play a role in antigen presentation to thymocytes. This can be of functional importance for establishment of the T cell repertoire. There are experiments in mice that show that deletion of certain Vβ TCR T cell families occurs through MLS antigens expressed by thymic B cells harbouring MMTV (*Marcos*).

TOLERANCE AND SELF ASSERTION

(A. Coutinho)

Tolerance and the gut microflora

Tolerance is considered usually in the context of autoimmune diseases and allograft rejection. The majority of antigens that are encountered every day are self-antigens. If we take into account that there are 10 times more bacteria than eukaryotic cells in the human body (*van der Waaij*) the interaction of the immune system with intestinal antigens may not be overlooked and should be viewed in the light of tolerance as well. When we are speaking of tolerance one should be aware of the fact that tolerance depends upon the read out system and parameters tested. Apparent unresponsiveness of the immune system is not a passive state but is maintained through an active and dynamic process.

Intestinal bacteria are important for physiological functions of the human body (vitamin K production, colonisation resistance, digestion of food products, differentiation of the gut epithelium, mucus induction, haematopoiesis, etc.). For further information on this topic see Old Herborn University Seminar Monograph 1: Microecology of the Human Digestive Tract. Meanwhile it is of crucial importance that strong immune responses against gut microflora antigens do not occur. The bacteria live in symbiosis with the host's immune system. On the other hand the immune system should be able to induce specific immune responses to (potential) pathogenic bacteria.

Patients with agammaglobulinaemia are believed to have a normal microflora, which may suggest that there is no role for IgA in the gut with regard to regulation of the microflora. However, despite this statement, 90% of the IgA plasma cells are found in the intestinal tract.

The important role of the intestinal microflora is also illustrated in GvHD models. Lethally irradiated mice engrafted with allogeneic bone marrow cells may suffer GvHD if the recipients are conventional, whereas GvHD is absent when recipients are germfree (GF) or have been totally decontaminated (*Heidt*, *Veenendaal*). Furthermore, GF mice have shown to respond differently to mouse related microflora compared to mouse unrelated (human) microflora. After oral association with microflora, mice have been found to respond with higher antibody levels to a significant higher percentage of bacteria present in the human derived flora as compared to those in the mouse derived flora (*Veenendaal*). However, it remains unclear whether selection of intestinal microflora organisms is mediated through the immune system.

With regard to autoimmunity the gut microflora also plays an important role. Neonatally thymectomised mice or nu/nu mice suffer lupus-like autoimmune disease when they are maintained

under conventional conditions. However when they are germfree, isolated and monoassociated with *E. coli*, or conventional but isolated, no lupus-like autoimmune disease will occur. Similar results have been found in NZB mice. These results suggest that environmental bacteria or antigens, which are absent in isolated animals, are most important in inducing lupus-like autoimmune disease in functionally thymus deficient animals, whereas the role of the autologous microflora may be of limited importance. On the other hand the intestinal microflora may also be protective for autoimmune disease as BB rats or Fisher rats become more susceptible for diabetes or arthritis respectively when they are germfree.

B cell tolerance

General remarks

Discussing B cell tolerance the following points should considered. It is obvious that the V_H gene repertoire is of importance. Other aspects that are related with tolerance are the control of effector function: Many immune activities are not destructive at the target e.g. even in the presence of autoantibodies target organs are not always affected. Finally, the dynamics of antigen and immune response play a role in tolerance induction. The immune system only responses to major changes in the antigenic concentration (including self). Indeed usually self-antigens are kept at a constant level. Moreover, when xenogenous erythrocytes are injected slowly over a 24-hour period no specific antibody response is seen. Autoantibodies in immune diseases are not at a constant level but fluctuate.

B cell repertoire and tolerance induction

Deletion of B cells and their immediate precursors occur at different levels. With respect to particular V_H gene antigens, cell kinetics data show that many cells die at the transition of pre B cell to newborn B cell and from newborn B cell to mature B cell. This deletion process is clearly associated with selection phenomena, which are either positive or negative. The observation that autoreactive B cells are always seen in the periphery implies that a presumed deletion process is not complete. There is however no evidence that this failure of deletion results in autoimmunity.

How to explain this presumptive deletion of autoreactive B cells (negative selection) in the BM together with production of autoantibodies in the periphery (positive selection to antibody secretion)?

1. It could well be that different ligand concentrations have different effects on the B cell:
   a. low levels of ligand may result in survival but no secretion of antibodies
   b. intermediate levels of ligands may result in activation of the B cell concomitant with differentiation to plasma cell formation leading to Ig secretion
   c. high levels of ligand may lead to activation of B cells without secretion. The highest levels may lead to cell death.

   According to this view, if low levels of ligand are encountered during the generation of B cells, this results in positive selection whereas the highest levels may lead to negative selection. Total absence of ligands leads to apoptotic cell death.
2. According to *Cooper*, cross-linking of B cells at different stages of their development may have different effects. This may well be due to differences in signal transduction mediated after binding of proteins at different cell stages after cross-linking with different ligands e.g. IgM. This explains why immature μ^+ B cells treated with anti-μ undergo capping, whereas

mature μ^+ δ^+ B cells will be activated. Whether capping at the stage of the immature B cells will result in apoptotic death has not been investigated so far. Thus peripheral B cells may require higher ligand concentrations to become stimulated compared to immature newborn B cells in the BM. Furthermore for a particular B cell high affinity ligation results in deletion whereas low affinity ligation stimulates the B cell. In this way high affinity autoreactive B cells are deleted in the BM whereas multireactive low affinity B cells are positively selected and subsequently become stimulated into plasma cells in case higher concentration of autoantigens are encountered in the periphery.

3. Another explanation for the production of natural autoantibodies is that a different lineage i.e. CD5 B cells is responsible for the production of these antibodies, while autoreactive conventional B cells are deleted.

IgG autoantibodies

It is now clear that autoreactivity is not restricted to IgM antibodies but can also be found among IgG. The V_H genes of these IgG autoantibody-producing cells are generally not somatically mutated. Thus probably these cells have not participated in a germinal centre reaction. The affinity of autoantibodies (which are predominantly of the IgG isotype) has no relation to the biological function. Furthermore, there is no correlation between the titre and the degree or even the presence of autoimmune disease. For these reasons one should avoid the term pathogenic antibodies. Instead they should be named "Disease Related Antibodies".

Intravenous IgG treatment of patients with autoimmune disease has a beneficial effect. Mechanisms to explain this may include:

- blocking of Fc receptors
- supply of Ig anti exogenous bacterial related antigens
- supply of antibodies against cytokines mediating inflammation
- direct effect on autoantibodies
- adjuvant effects
- etc.

Autoantibodies may play a role in the fine regulation of the endocrine system as well. Some antibodies mimic hormonal effects, whereas others inhibit. The key function of this system is to dampen down major hormone level deviations. SCID mice, which lack immunoglobulins, might for this reason be more susceptible to all kinds of environmental variations. In this way autoantibodies determine the robustness of the endocrine system.

T cell tolerance

Contact at the T cell receptor level takes the same amount of aminoacids (15-20) as compared to ligand binding of B cells. Still there are major differences between B and T cells: The thymus is more secluded than the bone marrow. Therefore tissue specific antigens might never reach the thymus. This implies that there must be extrathymic T cell tolerance.

Normally a full tolerance for epithelium exists, which is not based on deletion. MLR is mediated by skin antigens presented by bone marrow derived cells, but not by skin antigens presented by the skin itself.

There is no good evidence that defects in deletion of autoreactive T cells are related to autoimmune diseases. Still intra-thymic deletion mediated by superantigens is known to take place.

Anergy does not exist at the level of T cells or B cells (*Coutinho*). Intrinsic all cells are or remain able to respond and therefore one cannot speak of anergy, tolerance or absence of response in a definite way.