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SPECIAL ARTICLE

Characterzation of Immunological Memory Cells. A Minireview.

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SUMMARY : This paper reviews the information concerning immunological memory T and B cells. It shows that the existence of different or a single lineage of memory and naive cells is still a question. The recirculation pattern of memory cells is different from naive cells. A unique recirculation for memory T or B cells is suggested while the bcl2 gene expression possibly plays role in the longevity of memory cells. Various phenotypic markers are demonstrated only on memory or on naive cells; however, a reliable and convenient method for the detection of memory cells still needs to be explored.

In general parlance, memory means the ability to store and to recall something. The meaning is the same for immunological memory. Moreover, in immunology we usually consider that memory functions by shortening the time required for the positive detection of antigens and especially for the rapid increase in production of specific high affinity antibodies. What are the differences among immunological memory cells and other immunological cells? Do we have both B and T memory cells. How long can immunological memory cells survive? How do they circulate? Where are they located? How can we detect or demonstrate them? These are some interesting questions that this article will try to address. Many studies have been performed and many hypotheses have been proposed, however, they are somewhat inconclusive. This minireview considers some aspects of immunological memory cells according to (1) lineage, (2) longevity and recirculation pattern, (3) phenotypic markers expression, and (4) detection methods.

Lineage of immunological memory B and T cells

The existence of memory B cells has been well recognised for decades, contrary to memory T cells which are comprised of various subpopulations. Therefore, greater controversy exists concerning the role of memory T cells in the immune response than about the role of memory B cells. The first criterion which indicates the presence of memory B cells is the shortened time required for positive detection of specific antibodies with high or increasing affinity for a given antigen. The function of memory T cells is more complicated since they encompass subpopulations of T cells and they cause no change in immunoglobulin isotype as observed with memory B cells. Nevertheless, memory T cells do exhibit specific activities when tested *in vitro* in comparison to naive T cells[1] and there is evidence for an increase in frequency of precursor memory T cells associated with certain viral infections.[2]

There are at least two ideas concerning memory B-cell development. The first proposes that there is only one lineage for B cells that leads to either naive cells or memory cells. According to this theory, some cells differentiate and mature into antibody producing cells upon interaction with specific antigens while others are arrested in their differentiation at a certain stage and become quiescent until reexposed to the same antigen, after which they resume differentiation into antibody producing cells.[3,4] T-cell derived factors may be involved in B cell differentiation. For example, the lack

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of an appropriate T-cell factor at some stage of B cell differentiation may prevent development of antibody-producing cells. This would suggest the existence or release of qualitatively different suppressive factors from T cells upon antigenic stimulation.[4] The second theory for B-cell development proposes that there are different lineages of B cells which become antibody producing cells or memory cells. This concept is supported by the existence of a specific, phenotypic marker, J11D, which can be detected at varying levels on B cells by a specific monoclonal antibody. The speculation is that B cells which express high levels of the J11D marker upon stimulation with specific antigen will generate antibody producing cells while B cells which express low levels J11D marker will generate memory cells.[4-6] Other evidence suggests that the microenvironment in various parts of lymphoid germinal centers may lead to hypermutation of VH genes with high antibody affinity or to the generation of memory B cells.[7-10]

Information from viral infections and from carrier models demonstrates the existence of both T helper and T suppressor memory cells.[11, 12] Which T-cell lineage acts as effector/regulator or memory effector/regulator is controversial. Recent studies favor the idea of only one lineage of T cells based on the leukocyte common antigen phenotypic marker CD45 (CD45RA and CD45RO). The work shows that naive T cells contain CD 45RA while memory T cells contain the CD45RO isoform. Whether CD 45 isoforms can be interchangeable is under investigation. At present, some studies suggest that CD45RA-positive T cells can become CD45RO positive cells.[4, 6, 13-15] If proven correct this indicates that either CD45RA or CD45RO can be expressed on the same T cell at different activation stages or at different transition stages.

The question of different or single lineage of memory and naive B, T cells is still a question, The obscure condition of the development of memory and effector T cells, the question of whether effector cells are end cells or revert to memory cells are challenges for the immunologist.[16] To give answers to these questions, specific or unique phenotypic markers are necessary. Elucidation of how early in lymphocyte ontogeny, certain genes are expressed only on either naive or memory cell should give some useful answers.

Longevity and recirculation of memory B and T cells

The exact life span or half-life of lymphocytes (B and T cells) is still an open question. This lack of knowledge is due to limitations in techniques for following specific B or T cells.[17] However, the approximate life span of naive lympocytes is thought to be 3-4 weeks. Memory B or T cells are believed to be longer living cells, having life spans greater than 1 month. There is even a report of a 10 year life span for memory B or T cells[18] and there is a report of a life span as long as 75 years for immunological memory to infectious agents.[17] In fact, there are two opposing ideas concerning the longevity of naive and memory cells. According to one point of view, naive and memory B or T cells have the same half life but the permanence or consistency depends upon restimulation of naive lymphocytes at the specific antigen depot in follicular dendritic cells of the lymphoid organ to allow them to function as memory B or T cells.[6] According to another point of view, there is a difference in their half lives. This view derives from studies which demonstrate different recirculation patterns associated with the expression of various adhesion molecules on naive and memory B or T cells.[19-22] For example, the recirculation of naive lymphocytes is via the high endothelial venule from peripheral blood into lymph nodes, while memory lymphocytes enter lymph nodes via the afferent pathway. Thus, memory lymphocytes circulate from the peripheral circulation to tissue and then to lymph nodes.[23] There is also evidence for the existence of two types of memory cells, ie circulatory memory cells and non-circulatory memory cells.[24]

However, the recirculation patterns of naive and memory B, T cells have been shown more clearly to differ according to the expression of an adhesion molecule, L-selectin, as indicated by the observation that naive B, T cells express more L-selectin than memory B cells.[25] Nevertheless the recirculation pattern of memory T cells may be different from memory B cells because there is evidence that a carbohydrate molecule termed cutaneous lymphocyte associated antigen (CLA) the ligand for L selectin is expressed only on memory T cells.[26]

Antiapoptotic function of the bcl2 gene in B and T cell survival has been suggested. For example, it has been shown that upon antigen stimulation in the presence of interleukin-2 some cells die by apoptosis while some survive and function as memory cells.[27, 28] It is suggested that the product of the bcl2 gene plays a role in lymphocyte survival by influencing apoptosis.[29] Although there is no direct evidence to indicate differences in longevity between naive and memory cells there is indirect evidence for a role of the survival gene bcl2 in memory cells to give longer half lives of memory B and T cells than naive cells. Moreover, transgenic mice overproducing bcl2 have been shown to have longer lived memory B cells[30] while T cells in homozygous bcl2 deleted mice had a shorter life span than in bcl2 expressed mice.[31]

Phenotypic markers of memory B and T cells

Phenotypic markers expressed on the surfaces of lymphocytes are important for their circulation and activities. Naive and memory (B or T) cells certainly express surface

phenotypic markers and adhesion molecules differently. Some of the markers expressed differently are shown in Table 1. [4, 6, 32-37]

However, these are some of the phenotypic markers studied in both humans and mice which some are useful only for mice, for example, Mel14 (Leu 8) and J11D.

Table 1. Phenotypic markers express differently on naive and memory cells.

Phenotypic marker	Naive cell	Memory cell
CD 2	++	+++
CD 7	++	±
CD 10	-	+
CD 18/CD 11 a	++	+++
CD 21	++	±
CD 23	++	±
CD 25	-	+
CD 26	-	±
CD 29	+	+++
CD 30	-	++
CD 38	-	++
CD 39	++	-
CD 44	++	+++
CD 45 RA	+++	-
CD 45 RO	-	+++
CD 54	-	+
CD 56	-	+
CD 58	+	++
CD 69	-	±
CD 73	+	-
IL-2 R (p 75)	-	±
MHC II	-	+
Leu 8 (Mel 14)	++	+++
J11D	++	±

How memory B or T cells are detected?

Knowing whether memory B or T cells, and especially their specfic antigens are present is very useful. For example, they can help us know whether a test vaccine elicits a potent or wanted protection or not and they can provide help in diagnosis and treatment of diseases. Various *in vitro* methods or techniques can be used to identify or detect immunological memory cells. The princples for these *in vitro* methods are described here.

A. Methods for the detection of memory T cells.

1. Increased proliferation in response to specific antigen challenge *in vitro*. This can be detected using isotope labelled precursors (nucleotides or amino acids), autoradiography and blast cell staining, for example.[38]

2. Staining of specific phenotypic markers such as CD45RO, CD44 and IL-2R P75 which are receptors for IL-2. These are the most reliable markers for memory T cells at present. In addition, a recent study has indicated that different adhesion molecule subunit combinations (alpha 4, beta 1) in memory and naive T cells (ie heterogeneous for memory T cells, and homogeneous for naive T cells)[36] can also be used. Staining techniques include fluorescence, and immunochemical staining, when results can be obtained using a fluorescence activated cell sorter (FACS).[39]

3. Detection of cytokines, for example, IL-2 IL-4 and interferon (IFN) production.[40-42] In the biological assay, CTLL cell line proliferation can be used for IL-2 detection[43] or CT-4S for IL-4 detection. By gel hybridization (mRNA-cDNA dot blot hybridization) specific mRNA cytokines can be detected.[44]

4. ELISA method. The ELISA plaque assay and ELISPOT assay are techniques for the detection of memory T cells at the single cell level.[45,46] These techniques are sensitive and convenient.

B. Methods for the detection of memory B cells.

Two of the above *in vitro* methods for detection of memory T cells can be used for B cells also. These are:

1. Increased proliferation in response to specific antigen challenge.

2. Staining or detection of certain phenotypic markers such as CD45RO.

Other *in vitro* methods used only for memory B cells are:

3. Increased quantity or rapidly rising level of IgG after specific antigenic challenge. Various methods can be used. Examples are immunoelectrophoresis, radial immunodiffusion and ELISA.

4. Plaque forming assays for detection of antibody producing cells, eg ELISPOT. The advantage of the ELISPOT assay can be shown for the detection of both memory T and memory B cells. This technique can be performed to detect individual cytokine secreting cells for memory T cells or antibody producing cells at the single cell level for memory B cells; quantitation of memory T or B cells is possible.

Since the definite characteristics of naive and memory B, T cells are not completely elucidated, the detection of memory B, T cells using antigen non-specific methods such as detection for the expression of CD45RO, CD44, selectin or the extrusion of P glycoprotein substrate Rhodamine-123[47] should be performed in parallel as far as possible. The antigen specific

method seems to be reliable, but is time consuming. At the gene level, in parallel detection for certain gene such as bcl2 may increase the sensitivity of detection of memory B, T cells.

CONCLUSION

Immunological memory is an important characteristic of the immune response and it differs from neurological memory in various respects. Various studies have characterized memory B and T cells according to lineage, longevity, circulation pattern and phenotypic markers. The ability to know how to detect the presence of memory B or T cells is very useful for medical treatment and for research in biological and medical science. However, this will be successful only when the complete characteristics of memory B and T cells are known and when reliable and convenient methods to assay these characteristics are developed.

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