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CONFERENCE PROCEEDINGS

Proceedings of the Conference "Lymphocyte Development, Tolerance and Autoimmunity: Solved and Open Questions", Held at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, on May 12, 2011 (Wrocław, Poland)

Lymphocyte Development, Tolerance and Autoimmunity: a personal perspective

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Introduction to the Conference

Understanding the mechanisms of the immunological tolerance and autoimmunity remains a major challenge for researchers and clinicians struggling to free the society from several serious diseases (e.g., diabetes), which result from the disturbance of the regulatory mechanisms maintaining properly functioning immune system. Often these disturbances have their origin in impaired lymphocyte development, therefore, the prospect for the progress in fighting autoimmune diseases or breaking tolerance to the tumors is very much dependent on the better understanding of the mechanisms of lymphocyte development and their functional diversification.

In view of the breathtaking progress in these areas of research and a daunting number of new publications appearing everyday, the direct contact and first hand information from leading experts play a very important role in catching up with what is going on at the cutting edge of current studies.

Considering above, on 12 May 2011, 1-day conference was organized in the Institute of Immunology and Experimental Therapy under the auspices of the Polish Academy of Sciences with the participation of renowned immunologists whose contribution to uncovering the intricacies of lymphocyte development and understanding the mechanisms of tolerance and autoimmunity is known worldwide. Abstracts of their presentations are published below in this issue of "Archivum Immunologiae et Therapiae Experimentalis."

The leitmotiv of the conference: "solved and open questions" was meant to emphasize, that the effort to understand the function of the immune, like any other biological systems represents the continuous, never ending asymptotic process where the answer to the given question is as good as the number of new questions it generates.

As the scientist "about to retire" and a chairman of the organizing committee of this conference, who spent 40 years studying the development and heterogeneity of T lymphocytes as well as the mechanisms of their selection, I was asked to introduce the subject of the conference from my personal "pre-historical" perspective in which the conference speakers played a prominent role. Below is my brief and simplified account of the research, in which I was fortunate to participate and which by solving old and opening new questions contributed to the better understanding of the functional heterogeneity of mature T cells as well as of the basic cellular mechanisms responsible for the generation of self MHC restricted and self tolerant repertoire of antigen-specific receptors of T lymphocytes.

In early 1970s when I learned about T and B lymphocytes, little was known about their development, physiological function of MHC antigens responsible for the graft rejection was unknown (Klein and Shreffler 1972) and except the information that T but not B cells need the thymus (Miller 1961; Warner and Szenberg 1962), its role was very unclear. One of the important questions asked at that time—however trivial it may look from today's perspective—was, whether mature T cells known to kill virus infected cells and to help B cells to make antibodies represent one type of a bi-functional cell or belong to two functionally different populations. As a post doc I was at that time in Dr. Lloyd Old's laboratory at the

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Sloan-Kettering Cancer Institute in New York, where having access to thymus specific alloantisera (Boyse et al. 1968), we showed (Kisielow et al. 1975; Kisielow et al. 1976) that effector T cells belong to two different populations: killers and helpers (today we would call them regulators), which we identified and separated by means of anti-CD8 (then called Ly2) and anti-Ly1 (later replaced by monoclonal anti-CD4 (Dialynas et al. 1983) antibodies. This was the time when Susumu Tonegawa just explained the mechanism of somatic generation of antibody diversity (Hozumi and Tonegawa 1976; Tonegawa et al. 1974), and Rolf Zinkernagel and Peter Doherty discovered the phenomenon of MHC restriction (Doherty and Zinkernagel 1975). Naturally, these Nobel prize winning discoveries overshadowed our finding, which nevertheless also generated quite a number of questions and in addition provided means to approach them experimentally, means which even today are used as basic tools in clinical and basic immunological research. Extending our finding, Harvey Cantor and Edward Boyse later showed that CD4 and CD8 cells represent different lineages generated independent of intentional antigenic stimulation. In accord with their function, CD4 cells were shown to be restricted by class II and CD8 cells by class I MHC molecules (Cantor and Boyse 1975a; 1975b). Some of the important open questions awaiting for an answer, concerned the identity of the immediate precursor of CD4 and CD8 lineages and the nature of selection mechanisms responsible for the fact that mature T cells are useful for the organism, i.e. self MHC restricted and self tolerant, in other words, protective but not auto-agressive.

Fruitful studies in Harald von Boehmer's laboratory at the Basel Institute for Immunology using HY TCR transgenic mice played leading role in finding answers to these questions (von Boehmer 1990). These studies were made possible by long awaited identification of T cell receptor genes (Hedrick et al. 1984; Yanagi et al. 1984), the ability to clone antigen specific T cells (von Boehmer et al. 1979) as well as emerging technology of producing transgenic mice (Gordon and Ruddle 1983; Storb et al. 1984; Uematsu et al. 1988). Together with my PhD students, Wojciech Swat, Leszek Ignatowicz, and Arkadiusz Miążek, I had a chance to participate in these studies. The main conclusion from these studies was that CD4 and CD8 T cells are generated from double positive (DP) $CD4^+8^+$ thymocytes which undergo two types of selection: negative or positive (von Boehmer et al. 1989; von Boehmer and Kisielow 1990). Recognition of self antigen by DP thymocytes results in their deletion by apoptosis which represents the central mechanism of tolerance to self (Kisielow et al. 1988a; Swat et al. 1991), while interaction with MHC molecules in the absence of self antigen—as we provisionally and over-simplistically interpreted our results at first—is necessary to rescue DP thymocytes from programmed cell death and induce their differentiation into CD8 or CD4 lineage, depending on the class of MHC molecule recognized (Kisielow et al. 1988b; Swat et al. 1994; Teh et al. 1988). Learning how CD4 and CD8 T cells, which I and my colleagues identified 15 years earlier (Kisielow et al. 1975) was one of the happiest experience in my scientific life. With Arkadiusz Miążek we showed that the interaction with MHC molecules is necessary not only to induce positive selection but that the continual interaction of rescued DP thymocytes with MHC molecules is also necessary for survival of generated single positive thymocytes (Kisielow and Miazek 1995). Others showed that it is also required for long-term survival of mature T cells (Kirberg et al. 1997; Takeda et al. 1996; Tanchot et al. 1997).

Concerning the mechanism of central tolerance, the crucial question remained whether tolerance to the tissue-specific differentiation antigens expressed outside the thymus could also be acquired in the thymus? This question was positively answered by Bruno Kyewski and colleagues who demonstrated that extrathymic antigens are presented to developing thymocytes by specialized subset of thymic medullary epithelial antigen-presenting cells (Derbinski et al. 2001; Klein et al. 1998).

In parallel to the above studies, the avalanche of new questions was building up as a result of the realization of ever growing functional heterogeneity of CD8 and especially CD4 T cells among which $CD4^+Foxp3^+$T regulatory cells (Fontenot et al. 2003; Hori et al. 2003) took the center of the stage as the major peripheral force deciding whether tolerance or autoimmunity will result from the interactions between cellular and humoral elements of the immune system (Sakaguchi 2011). The contributions of Harald von Boehmer and Leszek Ignatowicz to deciphering the heterogeneity, biology, and specificity of this presently most admired cell of the immune system is described in their several highly cited papers (Apostolou and von Boehmer 2004; Kretchmer et al. 2005; Pacholczyk et al. 2006; Pacholczyk et al. 2007; von Boehmer 2005). The avalanche of the opened questions concerning the function and developmental relationship between various $\alpha\beta$ T cells (e.g. Th1, Th2, Th17, NKT)—not mentioning $\gamma\delta$ T cells—was for me overwhelming and thrown me back to the beginnings that is to the questions concerned with the evolutionary and ontogenic origin of lymphocytes and their early developmental stages, the field to which important and well-known contributions were made by Louis du Pasquier (Azumi et al. 2003; Chretien et al. 1998; Du Pasquier et al. 1979), Fritz Melchers (Kudo and Melchers 1987; Sakaguchi and Melchers 1986), Ton Rolink (Ceredig et al. 2009; Rolink et al. 1991; Rolink et al. 1999), and Hans Reimer Rodewald (Rodewald et al. 1992; Rodewald et al. 1994; Schlenner and Rodewald 2010).

My own interest in this area is the consequence of unexpected findings made together with my present

collaborators, mainly by Małgorzata Cebrat (Cebrat et al. 2005; Kisielow and Cebrat 2007). Searching for new genes downregulating expression during positive selection we identified third strongly evolutionarily conserved gene within the locus of recombination activating genes whose transcription starts in *Rag2* intron and runs through the whole *Rag* locus control region. We called this gene *Nwc*, which is an acronym of polish words meaning "very interesting" or "nobody knows what it is good for." What we found interesting is that in contrast to the lymphocyte specific *Rag* genes, *Nwc* is ubiquitously expressed but in lymphocytes, it is regulated by *Rag1* promoter while *Nwc* promoter is inactivated (Cebrat et al. 2005; Cebrat et al. 2008). As a result, in addition to the *Rag1* transcript, hybrid *Rag1/Nwc* transcripts are generated.

On the basis of these observations and considering more than 400 million years lasting association of *Nwc* with *Rag* genes as well as its unique, lymphocyte-specific mode of regulation, we proposed that *Nwc* transcription could interfere with *Rag* expression thereby contributing to their permanent inactivation in non-lymphocytes and to their negative regulation in lymphocytes (Kisielow et al. 2008). The interesting question is whether inactivation of *Nwc* promoter in immature lymphocytes could have anything to do with the lymphocyte-specific release of *Rags* from permanent suppression during evolution or ontogeny of lymphocytes? (Kisielow et al. 2008; Kuo and Schlissel 2009).

So far our efforts to obtain experimental evidence in favor of this hypothesis are unsuccessful, partly because as we found the structure and regulation of the transcription in this locus are far more complex and complicated than anybody expected (Laszkiewicz et al. 2011) raising other interesting questions, which may have implications for the evolutionary mechanisms responsible for lymphocyte development.

As it is clear from the abstracts of lectures presented below, the progress in unraveling the heterogeneity of lymphocytes and the complexity of the immune system continues at the fast pace and it is astounding that the difficulties to incorporate the downpour of all new data into the comprehensive picture do not slow down the sucessful application of this knowledge (Daniel et al. 2010; 2011a) as shown, for example, by the spectacular progress in developing the vaccine against type 1 diabetes (Daniel et al. 2011b). It is true that, for the time being, this success has been achieved only in the mouse model of this disease but this means that there is no reason not to believe that this should be possible to reproduce in humans.

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