AUTOANTIBODIES-ANTIGEN DRIVEN OR IDIOTYPICALLY INDUCED*

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AUTOPROTILÁTKY NAVODZOVANÉ ANTIGÉNOM ALEBO IDIOTYPOVO INDUKOVANÉ*

The current acceptable notion regarding autoimmune diseases is that autoimmunity and autoimmune diseases are autoantigen driven. *This pathogenetic mechanism is very much conceivable according to theories in classical immunology, namely, if there is a "reactive" antibody, it must have been generated upon immunization with an antigen. Definitely, a support to this notion is gained from the fact some of the autoantibodies do bind specifically to* autoantigens *(one can refer to them as* target *— thus assuming that the binding ligands are also the* driving *autoantigens). In some of the cases (e.g. acetylcholine receptor in MG or gpIIb in ITP) there is a rational pathogenetic mechanism to explain the autoimmune attack in the specific autoimmune disease. Further support to the (auto)antigen driven theory comes from sequence analyses of nucleotides of autoantidoby Igs structure (especially in the CDR3) demonstrating somatic mutation, thus pointing to an antigen driven mechanism.*

To explain the sudden loss of tolerance to self antigens a linear or conformational changes in the autoantigens were proposed. More complex explanations were given to presumed intracellular and intranuclear autoantigens (e.g. DNA, nucleosoures, PDH etc.). In those cases there was a prerequisite necessity to explain their exposure to the immune system. In each one of these intracellular autoantigens it has been proposed that they are exposed to the immune cells on the surface of the respective cells, thus being able to stimulate the immunoreactive cells on the one hand, and being targets to the immune effector cells and antibodies on the other hand.

In the current article I would like to propose that in some autoimmune diseases and especially in the multisystem non-organ specific ones, in which characteristic and specific autoantibodies to ubiquitous autoantigen were found (DNA/SLE, RNP/MCTD, PDH/PBC etc.), there is no driven *autoantigen. Furthermore, binding of these existing serum autoantibodies to a presumed ubiquitous autoantigen, not necessarily means that the specific, multiorgan damage, indeed is mediated via binding of the respective autoantibody (or autoreactive T cell) to the autoantigen.*

Let me say in the beginning, that in contrast to organ specific autoimmune diseases (e.g. MG, AIHA, ITP), the fact that thousands of diligent and smart researchers through-out the world worked for decades to delineate a specific autoantigen in the non-organ specific diseases and failed, does not lead somebody to think that there is not *an autoantigen. Furthermore the existence of binding specificity does not point to the induction or pathogenetic mechanism(s): Let's take SLE/DNA and anti-DNA autoantibodies as a case in point. In 1956, 4 groups of researchers studying SLE sera picked up DNA from the shelf and found a high binding capacity of the sera to dsDNA. Furthermore a clinical correlation was reported between serum titers of anti-dsDNA autoantibodies and SLE activity, alluding to their pathogenetic role. Yet, at the same very days, sera of patients with SLE were found to bind even more avidly to a synthetic polypeptide poly (dG-dC), a synthetic shelf antigens. If poly (dG-dC) would have been picked up as a* shelf *antigen in 1956 by the 4 groups instead of dsDNA as a self antigen we might have today titering the levels of anti-poly (dG-dC) antibodies rather than anti-dsDNA as a clinical parameter for SLE activity. In a more modern fashion I would say the same in regard to nucleosomes. In recent years many groups raised the possibility that it is not dsDNA the target (and patho-immunologic) autoantigen but rather nucleosome (DNA-histome complex). Some claim that the Farr assay by which we detect high avidity anti-dsDNA antibodies is only due to the binding of those antibodies to nucleosomes. So there was also a need to explain how nucleosome break self tolerance to themselves. And indeed it was proposed that increased apoptosis of cells in SLE exposes nucleosome of cell on the surface, thus "bombarding" the immune system. I would not be suprised that in few years alternative autoantigens will replace DNA and nucleosomes (e.g. laminin, fibronectin, ...).*

As mentioned above, despite few reports pointing to the ability of autoantibodies to penetrate cells (Immunology Today A. Segovia), there is no good explanation, how binding of anti-DNA (nucleosomes) to DNA or nucleosome leads to psychosis in SLE or to pleuritis, pericarditis or bone marrow aplasia, or how binding of anti-RNP to 68 kD RNP in patients with MCTD causes Raynaud's phenomenon, and last but not least, how reaction between anti-topoisomerase-I, leads to increased production of collagen in systemic sclerosis. I would stress at this stage that all these examples

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* Full text of lecture.

differ from the binding of anti-AcR antibodies to AcR in MG, or anti-RBC antibodies to RBC in AIHA, or anti-gpIIb antibodies reaction with platelet in ITP. Thus there is no reasonable explanation for the clinico-pathological manifestations even by the most specific autoantibodies (e.g. anti-PDH and PBC).

Hypothesis

Is it possible that there is no autoantigen? Is it possible that as with the story of the existence of natural autoantibodies, we were misled by historical concept? For decades the autoimmune theories were blocked by Paul Ehrlich's "horor autotoxicus". For years, the existence of natural autoantibodies was dismissed by Burnet's "forbidden clones" theory. Is it possible that because in the classical autoimmune diseases, such as autoimmune hymolytic anaemia, immune thrombocytopenic purpura, myasthenia gravis, Graves' disease, pemphigus, etc., there are well-defined autoantigens and the pathogenesis is well explained by the binding of the autoantibodies to the respective autoantigens, we believe that in any disease regarded as an "autoimmune", one should find an autoantigen as the immunogen?

Scientific support for the lack of an inducing autoantigen
(Summarized in 1—5)

The failure to induce SLE in naive mice by DNA immunization or passive infusion of anti-DNA antibodies led us to embark on a new approach to induce SLE. This approach involves active immunization of healthy strains of mice with the anti-DNA antibody emulsified in complete Freund's adjuvant. After 3—4 months from the boost the mice developed all the serological markers characteristic of SLE, associated with clinical findings including increased erythrocyte sedimentation rate (ESR), leukopenia, thrombocytopenia, and kidneys and CNS involvements. There were differences in the induction ability of SLE among the various strains of mice, and it seemed to be related to MHC-I.

Following immunization with Ab_1 (Id), the mice developed Ab_2 (anti-Id) and eventually Ab_3 which had autoatibody properties. Our experiments may indicate that by idiotypic stimulation one can upregulate the production of (natural?) autoantibodies. It seems clear that the information required to produce these autoantibodies is inherent in our immune repertoire (e.g. the immunologic homunculus). These stimulations are specific: when we immunize with anti-DNA, anti-La or anti-Sm antibodies, the mice develop the serological repertoire seen in patients with SLE (anti-DNA, RNP, Ro/La). When immunized with anti-cardiolipin or anti-phosphatidylserine, the mice developed anti-phospholipid antibodies lupus anticoagulant, and when immunized with anti-proteinase-3 (Pr-3) (cANCA) they developed anti-Pr-3 and anti-myeloperoxidase (MPO) antibodies. In all the above experiments the mice developed manifestations of the respective autoimmune disease. When they were immunized with anti-DNA antibodies, one could see increased ESR leukopenia, thrombocytopenia, proteinuria, and immune complex deposition in the kidneys with mesangial deposition of the same idiotype employed at immunization (i.e. 16/6 Id), leading to glomerular atrophy. When immunized with anti-phospholipid antibodies the mice developed thrombocytopenia and thromboembolism. When they were mated they suffered from low fecundity rate and increased fetal loss. The most remarkable phenomenon observed entailed the generation by these naive mice of all the autoantibodies detected in the sera of individuals with SLE, including-anti-dsDNA, anti-histones, anti-cardiolipin, anti-Ro/La and even anti-Sm. Many of those antibodies were immortalized by the hybridoma technique, which was even used on to induce a second generation of mice with the experimental model. Furthermore, the nucleotide sequence of some of the Abs-3 anti-DNA antibodies resembled the sequence of Ab-1 used for the first immunization (E. Mozes, personal communication). I would like to stress that the mice were not immunized with DNA, and we were dealing with completely healthy strains of mice that are not prone to autoimmunity. The latter experiment with cANCA teaches us much with regard to the necessity for an autoatigen in the induction of the autoimmune disease. Mice leukocytes (WBC) do not contain Pr-3 (A. Wiik, personal communication). To confirm the production of anti-Pr-3 by the immunized BALB/c mice we had to use either human leukocytes or purified Pr-3 from human leukocytes as a substrate. When immunized with cANCA the mice developed sterile microabscesses, arteriolitis, granulomas, kidney involvement associated with mouse cANCA and anti-endothelial cell antibodies. Thus we induced Wegener's granulomatosis (WG) in mice that do not have Pr-3. Furthermore, on some occasions the induction of the respective autoimmune condition was performed with autoantibodies derived from healthy subjects (e.g. natural autoantibodies). All these arguments are supported by several observations, suggesting the possibility that some autoimmune diseases may arise, not by autoantigen stimulation, but by some defects or destruction of natural serum inhibitors suppressing the effect of natural autoantibodies. Another possibility recently suggested is that some cross-reactive idiotypes are B-cell superantigens.

Conclusion

Thus, it seems conceivable, despite extensive and elaborates studies, that in several "autoimmune diseases" in which the role of the presumed autoantigen was not clarified, we should abandon the idea that the autoantigen used for the detection of diagnostic autoantibodies is the inducing agent (immunogen) of the disease. This is especially true with SLE and DNA, but probably also holds true for Wegener's granulomatosis and Pr-3, primary biliary cirrhosis and the ubiquitous enzyme pyruvate dehydrogenase, as well as in the series of other autoimmune rheumatic diseases with intracellular autoantigens.

If this is so, how do we envisage that such autoimmune diseases are induced in patients? In the three experiments detailed above we have induced three autoimmune conditions — SLE, antiphospholipid syndrome, and WG — following immunization in the footpads of naive healthy mice with the specific autoantibody emulsified in Freund's adjuvant. In all cases, the mice developed the disease-specific autoantibody (Ab_3 = anti-anti-autoantibody). We are aware that this experimental induction is dependent on the presence of adjuvant and intracutaneous (intrapedal) injection. We postulate that the "natural" analogue of our experimental models resides in the induction of antibacterial antibodies carrying "pathogenic" idiotypes in patients following infections. Indeed, we have already reported previously on the presence of increased titres of the 16/6 Id, a pathogenic idiotype pf anti-DNA anti-

bodies, in the sera of patients infected with Mycobacteria (pulmonary tuberculosis) and Klebsiella (pneumonia and urinary tract infections) and other Gram-negative bacterial infections. We recently summarized this relatioship between infection and autoimmunity. Thus, it is conceivable that infection may trigger autoimmune diseases by inducing antibacterial antibodies carrying the pathogenic idiotypes of autoantibodies (Ab_1). In the presence of the adjuvant effect (or superantigen?) contributed by the various bacteria themselves, these antibodies (Ab_1) may initiate — in a subject with the "proper" HLA and hormonal background — the cascade of idiotypic dysregulation demonstrated by us in the experimental models leading eventually to the generation of Ab_3, which may either by itself or via regulation lead to the overt clinical autoimmune condition. Our results support Cohen's "immunological homunculus" suggesting that even though any Ab_3 may be generated, we are aware of some selection that is responsible for the limited number of autoimmune diseases encountered in nature. Recently Pascual and Capra raised the possibility that some cross-reactive idiotypes and specifically cold agglutinins utilizing the VH h-21 gene segments may react as B cell superantigens leading to upregulation of many B cells. Interestingly enough our 16/6 Id was sequenced recently, and found to be encoded by VH 4-21.

In many cases the disease emerges many months (or years) following the infections and, therefore, the relationship to the infections is remote. According to this theory, there is a group of autoimmune diseases in which, although there is a specific autoantibody and autoantigen(s), the autoantigen and/or the autoantibody are not necessarily directly implicated in the tissue damage. Thus, we do not have to explain how anti-DNA antibodies induce pleuritis or cognitive impairment, or how anti-cardiolipin leads to migraine and livedo reticularis, or how anti-proteinase-3 antibody causes glomerular lesion. Furthermore, we do not need to show the presence of intracellular autoantigens on the surface of some target cells as a prerequisite for pathogenicity of the respective autoantibody. The lesions in this group of diseases may be induced wither by external antigen, or by some disequilibrium in the idiotypic network.

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PREDSTAVUJEME NOVÉ KNIHY

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Žijeme v dobe vedeckého pokroku, kde sa nad človeka a jeho jedinečnosť dostáva technický zázrak. Neuvedomujeme si, že bez génia ľudského myslenia by sa technický zázrak nekonal. Možno aj pre hodnotu jednej alebo niekoľkých najcennejších myšlienok zabúdame na celého človeka, objaviteľa, vedca, lekára, mysliteľa, umelca, alebo obyčajného diváka a celú jeho osobnosť skrývame za jeho jedno dielo, často vytrhnuté z kontextu jeho života, jeho trápenia, radosti alebo úbohosti. Z encyklopédií umenia, lekárstva, kozmonautiky a ďalších sa ani zďaleka nedozvedáme nič o ich vynikajúcich reprezentantoch — najviac možno vyčítať ešte z dobovej fotografie, úsmevu alebo zatrpknutosti vo výzore príslušnej osobnosti. A tak ako lúč prichádza kniha PhDr. M. Černouška, ktorý dokázal preraziť anonymitu encyklopédií a preniknúť za štít fotografií významných osobností a priblížiť ich zložité duševno, tok myšlienok, okolnosti často nie plné uznania a geniálnosti z iného zorného uhla — z pohľadu psychológa, ktorý nie je len objavný, ale aj vysvetľujúci (a pre mnohých uspokojujúci). Možno nazvať výsadné zaujatie pre vedu, pre výskum, pre umenie, pre poznanie za šialenstvo? Nedotýka sa genialita učencov šialenstva, keď v zápale poznávania zabudnú na svoje dieťa v smrteľnej agónii? Napriek tomu sa podľa nich dodnes nazývajú objavené tkanivá, či štruktúry v mozgu a oni nie sú synonymom nezodpovedného otca. Alebo významný prínos v určitej oblasti prekryje a odsunie do zabudnutia všetko nesprávne? Možno sa takto vykúpiť napr. z trestného činu? Asi nie. Nehraničí to so "šialenstvom doby" — asi áno. Autor si vo svojom metafyzickom pohľade, do ktorého uchvacuje čitateľa, predkladá poznatky, ale nesúdi a už vôbec nezatracuje. Rozmer pochopenia pre absurditu mu dovoľuje nadhľad nepovyšujúci význam chýb minulých nad význam dnešnej obmedzenosti.

Celé dielo hovorí o človeku, o človeku s obrovskými duchovnými možnosťami a obrovskými telesnými a duševnými nedokonalosťami. Kniha je napísaná spôsobom, ktorý je pre našu spoločnosť prínosom aj v tom, že psychiatriu stavia na pozíciu, ktorá jej patrí, na miesto vedy, ktorá človeku pomáha, lieči ho, poznáva ho a dovoľuje mu poznávať samého seba — do pozitívneho videnia. Tento pohľad v našej spoločnosti môže prispieť k odstráneniu diskriminačných predstáv o liečbe na psychiatrii, o postavení psychiatrie v systéme lekárskych vied. A aj za toto patrí autorovi naše uznanie.

Knihu odporúčam do pozornosti lekárov, študnetov medicíny, ale aj múdrych ľudí všetkých iných profesií a zameraní. Kniha je prínosom pre kritické myslenie, pre schopnosť vidieť zastreté, pre poznanie a rozpoznávanie. A to je dnes pre nás všetkých najvýš potrebné.

M. Bernadič