

The role of apoptosis in autoantibody production

Il ruolo dell'apoptosi nella produzione di autoanticorpi

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RIASSUNTO

L'apoptosi è un processo fisiologico che garantisce il ricambio cellulare; dopo l'apoptosi i residui cellulari vengono rimossi per fagocitosi. È stato dimostrato che l'alterazione di alcuni meccanismi coinvolti nell'apoptosi possono favorire l'autoimmunità. Ad esempio, un difetto nei meccanismi regolati da Fas in corso dell'ontogenesi linfatica può consentire la sopravvivenza di cloni autoimmuni; allo stesso modo, la mancata rimozione dei corpi apoptotici contenenti autoantigeni può attivare cloni autoreattivi preesistenti e risultare nella produzione di autoanticorpi.

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In normal conditions, the intracellular autoantigens reach the cell surface by apoptosis and are normally cleared by phagocytes without inflammation, nevertheless the lack of depuration of apoptotic material foster the autoantibody production in individuals genetically predisposed, equally defects in signaling, execution and malfunction of the apoptotic pathways may induce autoimmunity, in consequence apoptosis is another way of understanding the autoimmunity (1).

Origins of autoantibodies

Autoantibodies may be expressed early during B cell ontogeny; nevertheless the autoimmune B cells are controlled by two check points: One in the bone marrow, this take place before the B cell maturation, in this check point the autoimmune clones are eliminated by receptor cross-linking. Another check point take place in the periphery and the autoimmune clones are eliminated by neglect (2).

Genetic mechanisms

The rearrangements of immunoglobulin genes produce a high affinity immune response, that is similar in normal and autoimmune individuals;

nevertheless in autoimmunity has been reported an abnormal gene rearrangement encoded by an atypical reading frames of CDR3, such rearrangement produce segments enriched in arginine that result in autoantibodies such as anti-dsDNA (3). Autoantibody production mainly results of hypermutation, this mechanism is normally expressed as response against mutant pathogens and produce a huge number of immune receptors against an unlimited number of antigens; the hypermutation is also important in vaccination (4). In spite that autoimmune B cells are usually deleted in bone marrow, few B cells can be transformed in auto-reactive cells in the periphery by somatic hypermutation, that is the case of the residue in 35 position of the H chain, this switch the affinity of the anti-phosphorylcholine to anti-dsDNA antibody (5). In consequence the bacterial and viral infections may induce somatic hypermutations of the V genes and produce cross reactivity against self-antigens; this has been reported in patients with SLE (6, 7).

Epitope spreading

T or B cells can recognize new epitops from an original antigenic site without cross reactivity with the same or different molecules; this ability is called epitope spreading. In SLE the response against Sm ribonucleoprotein is a good example of sequential progression in epitope recognition, therefore the anti-Sm B/B' response is compro-

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mised with the first epitome and its close relative (second epitome enlarged by neighbor amino acids) which is also recognized by the same antibody, the third or fourth epitomes are also recognized by the same mechanism, in consequence different epitops from a unique antigenic structure can be recognized by a single antibody (8). In diabetes the anti-GAD65 and anti-IA2 antibodies are linked to epitope spreading. In endemic pemphigus foliaceus, autoantibodies against the ectodomain 5 of the COOH end of desmoglein 1 appear firstly in the preclinical phase of the disease, then by epitope spreading autoantibodies against the ectodomain 1 (residues 1-108) and 2 (109-221) of the NH3 end are produced and coincide with the clinical manifestations (9).

Pos-translational modification of epitopes

A steric modification of a protein may trigger autoantibodies; this is the case of the peptides that are citrullinated like the Sa antigen, which is specific of rheumatoid arthritis (RA). Sa is a citrullinated vimentin of 50 kDa and is present in rheumatoid synovial; Sa seems to be a clue of a causal elusive

event of arthritis; interestingly the enzymes peptidyl-arginil-deiminases 2 and 4 that transform the arginine in citrulline, are both present in the synovial of patients with rheumatoid arthritis but not in normal synovial tissue (10, 11).

Molecular mimicry

Some viral proteins may cross react with human intermediate filaments (12); additionally some molecules like the lysogangliosides and the N-acetylglucosamine from the Streptococcus A group which are major epitops in rheumatic fever, are clinically linked to the Sydenham chorea and to rheumatic cardiomyopathy (13). Another example of molecular mimicry is the anti-phospholipid syndrome caused by antibodies against beta 2 glycoprotein I (b2GPI); the possible link of an anti-phospholipid antibody and infections is attributed to the hexapeptide TLRVYK shared by b2GPI with microorganisms such as Haemophilus influenzae and Neisseria gonorrhoeae (14, 15). Chemicals like the milk butirophylin (BTN) may produce cross reactivity with the extra-cellular NH3 domain of the oligodendrocyte myelin (MOG); in multiple scler-

Table 1 - Some autoantigens cleaved by proteases. Adapted from Utz et al. (113)

Disease	Autoantigen	Cleavage site	Protease
Autoimmune Hepatitis	Actin	LVID ₁₁ , ELPD ₂₄₄	C1
Coeliac Disease	Transglutaminase	Various	3
MCTD	U1-70 kD	DGPD ₃₄₁ /LGND ₄₀₉	3/GB
Overlap Syndrome	DNA-PK	DEV ₂₇₁₂ VGPD ₂₆₉₈	C3 GB
Poly/Dermatomyositis	Alanyl tRNA synthetase	VAPD ₆₃₂	GB
	Histidyl tRNA synthetase	LGPD ₄₈	GB
	Isoleucyl tRNA synthetase	VTPD ₉₈₃	GB
	Mi-2	VDPD ₁₃₁₂	GB
	PM-Scl (overlap syndrome)	VEQD ₂₅₂	GB
Scleroderma	CENP-B	VDSD ₄₅₇	GB
	Fibrillarlin	VGPD ₁₈₄	GB
	hnRNP C1 and C2	Various	C 3, 6, 7
	PM-Scl	VEQD ₂₅₂	GB
	RNA polymerase I and II	ICPD ₄₄₉ /ITPD ₃₇₀	GB
	Topoisomerase I	DDVD _{146r} , EEED ₁₇₀	C3
	SR protein kinase	PEDD ₁₂₃ /IEAD ₁₅	C 3, 6/GB
Sjogren's disease	NuMA	Various/VATD ₁₇₀₅	C 3, 4, 6, 7, 8/GB
	α -Fodrin	Various	Caspases
	UBF/NOR-90\	Various/VRPD ₂₂₀	GB
	Vimentin	IDVD ₂₅₉	C 1, 2, 3, 8, 12
SLE	Ki-67	VCTD ₁₄₈₁	GB
	Ku-70	ISSD ₇₉	GB
	La	DEHD ₃₇₁ , DEHD ₃₇₄	C 2, 3, 8, 9
	Lamin A, B	VEID _{230r} , EEID _{448r} , VEID ₂₃₀	C 6, ?, 6
	PARP	DEV ₂₁₆ /VDPD ₅₃₆	C 1, 2, 3, 6/GB

C = Caspases, GB = Granzyme B.

rosis the presence of anti-BTN antibodies in cerebrospinal fluid suggest a shared epitope of MOG/BTN₇₆₋₁₀₀ with myelin; this data suggest that a molecule present in the diet may stimulate the pre-existing autoimmune clones (16).

Autoantibodies are clinical markers

Autoimmune diseases are widely distributed; they are mediated by antibodies and reactive cells to self antigens. Clinically the diseases are classified in systemic diseases such as systemic lupus erythematosus and organ specific like the pemphigus. Autoantibodies are markers of autoimmune disease and recognize specific antigens; in some instances these autoantibodies are pathogenic because they participate directly in tissue damage; in other instances are predictors of the clinical outcome (17-19). Additionally, the autoantibodies may help to study the molecular modifications of self antigens induced by apoptosis (20). Autoimmune diseases affect more than 10 millions of Americans, among the "top ten autoimmune diseases" are: Graves disease, rheumatoid arthritis, Hashimoto thyroiditis, vitiligo, type 1 diabetes, pernicious anemia, multiple sclerosis, glomerulonephritis, systemic lupus erythematosus and Sjögren's disease, they have an autoantibody marker (21). Diverse autoantibodies may recognize native autoantigens and/or molecules modified by apoptosis (Tab. I).

Apoptosis

Is a physiologic process that guarantees the cellular exchange and produce cellular changes such as tightening, membrane blebbing, DNA fragmentation and mitochondrial release of cytochrome C. Apoptosis and necrosis are different because the latter is passive and induce cell disruption with inflammation; meanwhile the apoptosis is active and is developed without inflammation and not release of intracellular material. Along life a healthy individual produce 1×10^{20} Kg of cellular debris that is cleaned by phagocytosis. During apoptosis the intracellular self-antigens are translocated to cell surface and become available to pre-existing autoimmune repertoires (22, 23).

Extrinsic pathway

Involves the tumor necrosis factor family receptors (TNFR) led by Fas (CD95/Apo-1), they activate a signal transduction that recruits and activates the caspase-8, that in turn cleaves of the downstream effector's caspases. The intrinsic or mitochondrial pathway is triggered inside of the

cell by direct caspase activation or by intracellular changes that release pro-apoptotic mitochondrial factors, this activate the caspase 9 that in turn trigger the effector's caspases inside the apoptosome. The molecular link between both pathways is Bid which is a member of Bcl-2 family that is translocated to mitochondrial membrane by the cleavage induced by Caspase 8; Bid produce pro-apoptotic changes (24). The proteins involved in apoptosis are: Cell surface receptors and intracellular proteins including caspases.

Receptors of death cell

Includes eight main members:

1. Tumor necrosis factor receptor or DR1 (CD120a, p55 and p60).
2. Fas or DR2 (CD95, APO1).
3. DR3 (APO-3, LARD, TRAMP, and WSL1).
4. TNF-related apoptosis-inducing ligand receptor or TRAILR1 (DR4, APO 2).
5. TRAILR2 (DR5, KILLER, TRICK2).
6. DR6.
7. Ectodysplasin A receptor or EDAR,
8. Nerve growth factor receptor.

The extra cellular domains of death cell receptors contain a variable number of cysteine-rich domains, which are activated by their respective ligands: for instance TNF α activate TNF-R1, FasL and TL1A activate Fas and DR3 respectively, TRAIL activates DR4 and DR5, the ligand of DR6 remain to be identified. The intracellular "death domain" (DD) of each receptor is required for the signaling transduction, because it recruits different molecules that activate the caspases cascade. The death ligands interact simultaneously with the decoy receptors (DcR) and with osteoprotegrin (OPG), but do not transduces signals (25-33).

Caspases

This family of intracellular enzymes contain cysteins in a highly conserved active site (QACRG), this pentapeptide cleave at the aspartic residues of the target protein, working like "molecular scissors". The caspases are produced as proenzymes; they contain an amino-terminal caspase recruiting domain (CARD) and two subunits: one large (p20) and another small (p10), the caspases associates in a heterodimeric active form, and then the CARD prodomain is removed (34). The active caspase 3 and caspase 6 cleaves the death substrate poly(ADP-ribose) polymerase (PARP) which is a DNA damage signal protein, that interacts in a non-covalent fashion with different proteins altering

their functions (35, 36). In *Drosophila* there are 7 different caspases, meanwhile in mammals there are 14. The caspases with large prodomains are 1, 2, 4, 5, 8, 9, 10, 11, 12 and 13.

Death receptor signaling complex

Functionally the receptors are classified in two groups: the first group comprises a death-inducing signal complexes (DISCs) formed by Fas, TRAILR1 or TRAILR2 which recruit the same DISC machinery (FAAD), pro-caspase 8/10 and FLIP_{L/S}; the death effector's domain (DED) of FAAD work together with the DED of procaspase 8 and 10 and FLIP, this complex activates caspase 8 that in turn cleaves procaspases 3, 7 and 6; by this way the Lamin A, Actin, Gas2, Fodrin, Gelsolin, PKC, PARP, ICAD and Rb are attacked, this cleavage results in cell shrinkage, membrane blebbing and DNA fragmentation (37). There are two types of Fas signaling, the type I is of high performance in the DISC formation and produce large amounts of active caspase 8, meanwhile the type II is a low performance in the Fas-DISC complex formation and low caspase 8 level, this is compensated by a release of cytochrome c through a truncated Bid, cleaved by caspase 8.

The liberation of cytochrome c results in the apoptosome formation that is followed by the activation of procaspase 9 which cleaves the effector's caspases (33, 38-40). The second group of receptors engages TNFR1, DR3, DR6 and EDAR which transduce apoptotic signals by two different signaling complexes: The complex I formed at the membrane and includes: TNFR1, TNF, RIP (receptor-interacting protein), TRADD (TNFR-associated death domain protein), TRAF-1/2 (TNFR associated factor); the complex I initiate the NF- κ B activation by IKK recruitment and JNK activation and depends of a TRAF-2 mechanism without FAAD, and the activation of NF- κ B transcription leads the expression of survival genes (33, 41). The complex II implies the traddosome formation, therefore the amount of FLIP determine whether caspase-8 is activated (42, 43).

Mitochondrial pathway

Cell death signals of the intrinsic pathway are leaded by proteins of the Bcl-2 family, such as the cytochrome C, Smac/Diablo and others that activate the caspases cascade. The procaspase 9 is recruited and activated by the heptameric "apoptosome" which is formed by the released cytochrome C that bind monomers of Apaf-1 and catalyzes the acti-

vation of caspase 9, this activates the effector's caspases-3, 6 and 7 leading apoptosis (44-46).

Clearance of apoptotic material

After apoptosis the cellular remains are quickly cleaned by phagocytes, which recognize tags on the surface of apoptotic cells; the phosphatidylserine (PS) is one of them and it is negatively charged in the inner membrane bilayer, by apoptosis PS is translocated to cell surface and interacts with phagocytes and macrophages. Another tag is the lysophosphatidylcholine that operates as a soluble phagocyte attractant (47). PS is identified by phagocytes through the phosphatidylserine receptor (PSR) that trigger a signal transduction. Other receptors involved in recognition are: α V β 3 integrin, CD36, CD68, CD14, ABC1 (ATP binding cassette transporter), β_2 -GP1R (β_2 -glycoprotein 1 receptor), β_2 -integrin receptor and Calreticulin. There are other ligands and receptors that participate in the "phagocytic synapse" these include C1q, β_2 -GP1, MFG8, thrombospondin 1 and oxidated LDL. The pentraxines such as CRP and seric amyloid (SAA), as well as the complement fractions C4 and C2 are involved in the clearance (48).

Autoimmunity and apoptosis

Apoptosis is essential during the lymphoid ontogeny; the immature T cells lacking of functional T-cell receptors (TCR) "die by neglect" because they express low levels of Bcl-2, this activate the mitochondrial pathway (49). In addition, the TCR with strong self reactivity are eliminated centrally by the Fas pathway (50). Autoimmune B cells under development may also die by apoptosis which is induced by BCR cross linking and the lacking of IL-4, CD40L and BAFF co-stimulation. In the periphery the B cells requires at least two signals to live:

1. The intrinsic expression of BCR on cell surface
2. The signaling induced by the cytokine BAFF; the BAFF transgenic mice increase importantly the number of mature B and effector's T cells, and develop autoimmune-like manifestations like high levels of rheumatoid factor, circulating immune complexes, anti-DNA autoantibodies, and immunoglobulin deposition in the kidneys (51, 52).

Defect of the Fas pathway

Mutations of Fas receptor and/or FasL, and the kinases involved in signal transduction of Fas have been implicated in autoimmunity. Canale and

Smith described a clinical picture in children with non malignant lymphadenopathies associated with autoimmunity; this clinical entity was called ALPS (53). After clinical description, the molecular basis for this disorder was demonstrated in MLR *lpr* mice; these animals develop glomerulonephritis, lymphadenopathies, hypergammaglobulinemia and antinuclear antibodies (54). Other mutants such as the *lpr^{cg}* and *gld* mice, develop deficiency in Fas expression by a missense mutation of the extra cellular domain of FasL, this mutation abolish the ligand function (55). The ALPS patients exhibit clinical and functional variants in the expression of Fas (56-59), this defect up-regulates CD28 that produce activation and proliferation of autoreactive T cell clones, that in turn activate the Fas-deficient B cells which produce autoantibodies (60); nevertheless a deficiency in autoimmune clonal elimination by the Fas pathway seems to affect more the peripheral tolerance rather than the central control, because the negative selection into the thymus is not impaired in the *lpr* mice (61).

Defect of the mitochondrial pathway

The engagement of B cell receptor (BCR) in developing o mature B cells, prevents the B cell autoimmunity in absence of T help; however the absence of the pro-apoptotic Bcl-2 family member Bim, make refractory the B cells to commit apoptosis by BCR ligation, in consequence this deficiency allows the survival of autoimmune clones that may result in a SLE-like disease (62, 63).

Defect in clearance

The presence of molecules from innate immunity on the surface of apoptotic cells, enhance the cleaning of apoptotic material by macrophages. Patients with autoimmune diseases associated to complement deficiencies, are impaired to clean properly the apoptotic material (64, 65). For instance the C1q deficiency result in SLE; the C1q-deficient (*C1qa^{-/-}*) mice develop spontaneous antinuclear antibodies with glomerulonephritis, therefore the presence of huge amounts of glomerular apoptotic bodies precede any clinical manifestation, it seems appear that the abnormal presence of autoantigens in glomerulus's is determinant for the formation of immune complexes *in situ* (66-68); the coating of apoptotic cells by the C3bi, CR3 and CR4 fractions is necessary for the engulfment of apoptotic material (69). Not only deficiencies in the complement fractions may induce autoimmunity, also some mutations in the complement re-

ceptors are associated to SLE, for example the CR1, CR2 that are crucial for the cleaning pathway as demonstrated NZM2410 mice, furthermore the *Cr2* gene deficiency confer susceptibility to SLE (70). Other molecules like the mannose-binding lectin (MBL) that behave structural and functionally as C1q and bind apoptotic cells in a late stage of apoptosis are important for cleaning the apoptotic material, but not determinant for autoimmune development, MBL null mice that develop defects in clearance do not develop spontaneous autoimmunity, lymphoproliferation, or germinal centers expansion as expected, rather they develop increased numbers of B1 cells (71, 72). A variant polymorphism of MBL gene produce low amounts of MBL and is associated to dermatomyositis (73). In summary, the insufficient clearance of apoptotic material may result in a high concentration of native or modified antigens which are potential targets of autoimmune clones.

Apoptosis induces nucleic acids and ribonucleoproteins fragmentation

The chromatin fragmentation is the hallmark of apoptosis; this is leaded by caspase-3, that activates the DNA fragmentation factor (DFF) or caspase-activated DNase (CAD) that in turn cut the DNA in ~200 bp fragments. Another endonuclease G (Endo G) contribute to DNA degradation; both endonucleases attack the chromatin and yield 3'-hydroxyl groups of 50-300 kb cleavage products, and 5'-phosphate residues at the level of internucleosomal DNA fragmentation (74). After fragmentation the apoptotic DNA bind the phosphatidylethanolamine and phosphatidylserine of apoptotic membranes and become accessible to APC throughout blebs, it seems appear that the cleaved DNA induce maturation of bone marrow-derived dendritic cells. Additionally, the DNA methylation as well as the IFN-alpha participation are critical for anti-DNA production (75-79).

Apoptosis affects the nucleolus and nucleoplasmic RNP containing structures

They suffer significant changes during apoptosis (80); this changes involve the rRNA degradation by the Fas pathway, which occurs at the 3'-end region of 28S rRNA, therefore the caspase-3 expose part of the large subunit; additionally, different ribonucleases caspase dependent (CARs) are activated and produce extrusion of the nuclear RNP structures to the cell surface; also during apoptosis mRNAs are cleaved previously to eIF4GI and

other initiation factors, this cleavage may result in anti-nucleolar antibody production in diseases like scleroderma (81-85). Patients suffering of the autoimmune mixed connective tissue disease produce huge amounts of anti-U1-70RNP autoantibody, and this ribonucleoprotein is one of the most affected by apoptosis, therefore the caspase 3 cleaves U1 70 RNP at position ³³⁸DGPD₃₄₁, and the fragmentation increases its antigenicity (86-88); interestingly the anti-RNP autoantibodies recognize preferentially the apoptotic epitope of U1-70K (89).

Additionally it has been demonstrated that the truncated fragments of U1A and U1C are more reactive to anti-RNP antibodies (90). Other small ribonucleoproteins like the Sm-F peptide is modified by caspase 8 and by proteases of the mitochondrial pathway (91). The cleavage of U1 snRNA occurs at the 5'-side of either Ψ 6 or Ψ 7 in the single-stranded 5'-end of the snRNA, the flanking pseudouridines are involved in the cleavage sites. Other small U2, U3, U4, U5 and U6 snRNA molecules are not modified by apoptosis (92). The heterogenous nuclear ribonucleoproteins (hnRNP) are cleaved by caspases, resulting fragments of 32, 29, and 16 kDa (93); other RNA binding proteins modified by apoptosis include hnRNP A1, hnRNP C1/C2, FUSE binding protein, nucleolin, Rho GDI 2, and the transcription factor BTF3 (94). The small hYRNAs complexed with Ro and La are degraded by caspase 3; such degradation is inhibited by Zinc and by caspase inhibitors and by Bcl-2 (95).

In apoptosis, Ro remains bound to a highly conserved region of the hYRNA, such binding protect of degradation to one 22 to 36 nucleotides domain; La ribonucleoprotein which is also associated with the hYRNAs, behave in similar manner and protects 27 to 36 nucleotides of the hYRNA (92, 96), apparently Ro60 protein is not cleaved by caspases or granzyme, however by apoptosis Ro suffer self-aggregation (97, 98). The ribonucleoprotein La is cleaved by caspase 3 at Asp-374 in the COOH terminus (99). At least 420 autoantigens are cleaved by apoptosis among them are: DNA-PK, NuMA, topoisomerase I, Fordin hnRNP C1 and C2, La, Lamin A, NuMA, PARP, Topoisomerase I and Vimentin among others (97, 99, 100). Table I. Another way of autoantigen cleavage is induced by the cytotoxic lymphocytes, therefore the activated CD8 cells (CTLs) and NK cells release toxic granules containing granzyme B; during viral infection the cytotoxic cells may release

granzyme B, this also may occur in cancer; the granzyme B cleaves and activates several pro-caspases and other proteins in different sites than caspases (99-102). Among autoantigens cleaved by granzyme B are: DNA-PKcs, NuMA, Histidyl tRNA synthase, Alanyl tRNA synthase, CENP-B, Fibrillarlin, Ku-70, La, Mi-2, PARP, PM-Scl, PMS1 -2, RNA polymerase I-II, Topoisomerase I and U1-70 kD (98,103).

Phosphorylation induces autoantigen cleavage

The splicing factors (SR) are phosphorylated during apoptosis, for instance the SR, ASF/SF2 and SC35 factors which are associated with the spliceosome and the splicing factor kinases (SRPKs), are activated by sub-lethal stress, this trigger an alternative splicing of anti-apoptotic mRNAs bcl-xl and Ich1 isoforms, nevertheless by lethal stress the SRPK1 activity is shut down by caspase mediated proteolysis, therefore the regulation, localization and possible modifications of the SR autoantigens depends of the stress level (104, 105). The cellular stress induces association of diverse phosphoproteins with the small nucleolar RNPs, such association may trigger the autoantibody production in diseases like scleroderma, were autoantigens like the snoRNPs form part of a phosphoprotein complex composed by fibrillarlin and the serine/arginine (SR) splicing factors such as SRp40, the phosphorylated complex may trigger an antibody response against fibrillarlin (106).

The IL-1B converting enzyme (ICE) family proteases cleaves the poly (A) ribose polymerase and the U1 70-kD snRNP, also cut the DNA-dependent protein kinase (DNA-PK) and the nuclear mitotic apparatus protein and the Lamin B; ICE protease operates in the effector's phase of apoptosis cleaving phosphoproteins; therefore the cleavage and phosphorylation are not necessarily mutually exclusive events, however it is possible that the phosphorylation of serine in autoantigens may promote the cleavage mediated by ICE-like protease in domains of DNA-PK, this is the case of U1-70 kD, nuclear Lamin B and UBF/Nor-1 (107).

Other targets of apoptosis are the Golgi apparatus proteins: Giantin/macrogolgin/GCP372, golgin-245/p230, golgin-160/GCP170, golgin-95/GM130, golgin-97, and golgin-67, they have coiled-coil domains (108-110), and the Golgin-160 contains at the N-terminal head domain several putative binding domains, and regulatory motifs and phosphorylation sites. It has been demonstrated that a caspase-dependent cleavage of the golgin-160 head

Table II - Examples of chemically induced autoimmunity.

Chemical	Disease	Autoantibody	Mechanism involved	References
Procainamide	Lupus	Anti-histone	Apoptosis, DNA metylation	117-119
Isoniazid	Lupus	ANA, anti-histone	Slow acetylator genotypes	120-122
Methylodopa	Lupus	H2A-H2B-nDNA, anti-erythrocyte	Lupus	123
Hydralazine	Lupus	ANA	ERK pathway inhibition	119, 124, 125
Chloropromazine	Lupus	ANA	Apoptosis	126, 127
Quinidine	Lupus	ANA, anti-chromatin	Apoptosis	118, 128
Sulphasalazine	Lupus	ANA	Slow acetylator genotypes	129, 130
Carbamazepin	Lupus	ANA, Anti-histone	Increase of B cell activity	131, 132
Phenytoin	Hepatitis	Anti-CYP3A	Drug-metabolizing enzymes	133
	IgA bullous dermatosis Lupus	Anti-histone	Moderate apoptosis inductor Low endogenous levels of dehydroepiandrosterone	134, 135 136
Propylthiouracil Methimazole	Lupus, vasculitis,	ANA, anti-dsDNA, ANCA	Apoptosis	137, 138
D-Penicillamine	Autoimmune syndrome	ANA Anti-epithelial antibodies	Cell stress	139
Chlorothiazide and hydrochlorothiazide	Cutaneous lupus erythematosus	Anti-Ro		140
Beta blockers	Lupus	ANA, anti-histone, anti-ssDNA antibodies	Polyclonal stimulation of lymphocytes	141,142
Valproic acid	Lupus	anti-dsDNA ANA, antiphospholipid antibody	Metabolite valpromide	143, 144
Terbinafine	SCLE	ANA, anti-Ro, anti-histone (H1 and H3)	HLA-B8,DR3 haplotype;	145, 146
Mercury	Scleroderma	Anti-Fibrillarin-U3	Apoptosis	147, 148

domain occurs rapidly after apoptosis induction, and the caspase cleavage at Asp139 site depend of a previous phosphorylation carried out by MLK3 (111).

Dephosphorylation occurs less frequently during apoptosis, for example La autoantigen is dephosphorylated at serine 366, and it seems appear that the kinase inactivation and the up-regulation of phosphatase A2 may produce dephosphorylation of La (112, 113). Another group of autoantigens dephosphorylated by apoptosis is the ribosomal proteins; anti-P ribosomal autoantibodies recognize all P proteins dephosphorylated by apoptosis, such process modify the conformational determinants of P proteins at the COOH end, this conformational modification foster the autoantibody production (114).

Autoantibodies and pharmaceutical agents

Autoimmunity can be triggered by the use of different pharmaceutical agents, also the chronic intoxication with metals have been ascribed to chemical induced autoimmunity, the molecular mechanisms that participate in this process are: 1. The inhibition of the DNA methylation may activate the

T cells. 2. Production of reactive metabolites that interfere with the tolerance. 3. Activation of antigen presenting cells (APCs) by drugs or metals 4. Drug induced apoptosis, as is the case of the chemotherapy increases the cellular debris and may trigger the autoantibody production (115). The clinical association between drugs and autoimmunity was reported since 1945, a patient who developed lupus after treatment with sulfadiazine; then in 1953 some cases of lupus related to the use of hydralazine were reported (116), subsequently other publications pointed out the role of hydralazine in triggering the antinuclear antibody production (117). More than 80 drugs may induce autoimmunity; most of them induce lupus and other diseases. Drugs include anti-hypertensive, anticonvulsivants, diuretics, anti-thyroidal drugs, antiarrhythmics, anti-tumoral therapy and by chronic intoxication with heavy metals (117-152) (Tab. II).

In summary, the tolerance would be shut down by diverse factors which would drive the autoantibody production in individuals genetically predisposed.

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SUMMARY

Apoptosis is the physiologic process that guarantees the cellular exchange; after apoptosis the cellular remains are cleared by phagocytosis. In autoimmunity, some mechanisms in apoptosis fail and may result in disease. For instance, a failure in the Fas pathway during lymphoid ontogeny may allow the survival of autoimmune clones; equally the lack of clearance of apoptotic corps containing self-antigens may activate pre-existent auto-reactive clones and may result in autoantibody production. The role of apoptosis in autoimmunity is reviewed.

Key words - Apoptosis, autoimmunity, autoantibodies, caspases, Fas/FasL.

Parole chiave - Apoptosi, autoimmunità, autoanticorpi, caspasi, Fas/FasL.

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