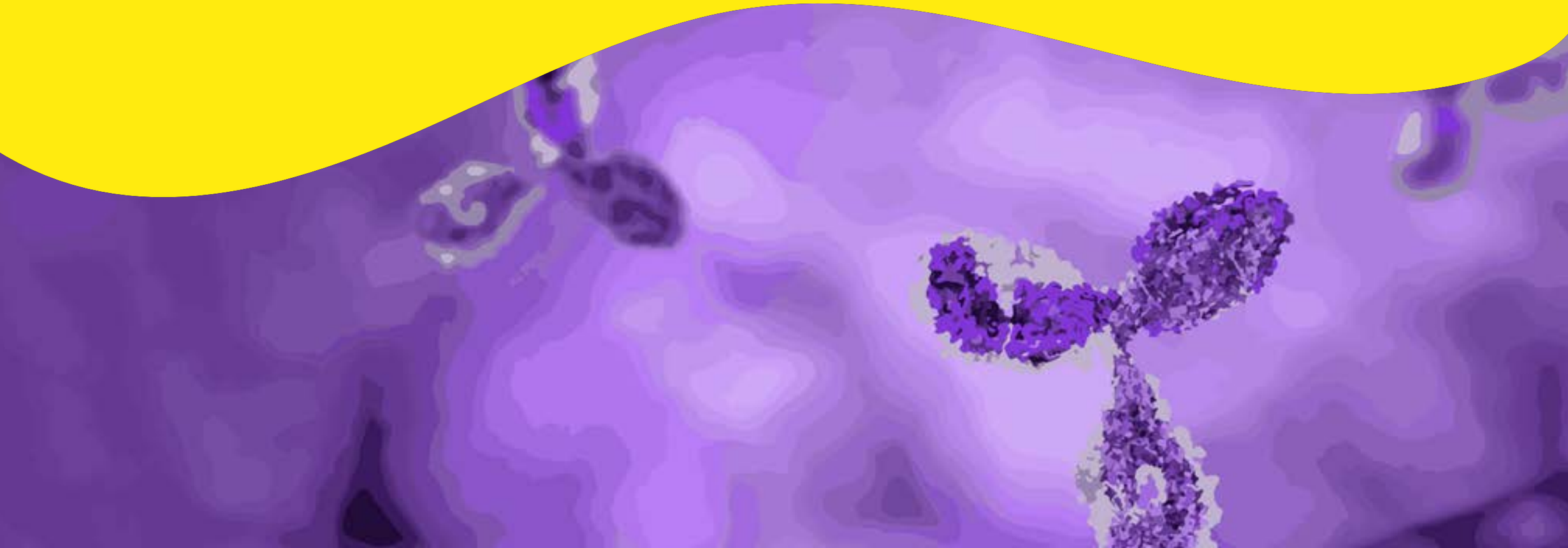
Autoimmunity

Guide to autoimmune diseases and therapeutic strategies



PURPOSE AND SCOPE

What to expect?

Welcome to this Autoimmune Diseases Booklet, a document to help scientists and researchers appreciate and navigate the diversity of the cell types and molecular pathways associated with autoimmunity, and the dysregulated immune responses associated with autoimmune diseases. We hope the visuals provided in this document will shed light on and clarify these otherwise complex mechanisms.

Among the over 80 autoimmune diseases reported so far, this document focuses on Rheumatoid Arthritis, Systemic Lupus erythematous, Inflammatory Bowel Disease, Multiple Sclerosis, and Type I Diabetes.

You will find three separate sections:

The first groups the main facts understood about the pathogenesis underlying each of the autoimmune diseases listed above, with a short description of its associated symptoms and complications, as well as risk factors and key cellular and molecular involvements.

The second section dives more deeply into the cellular and molecular mechanisms generally associated with the diseases.

The last section gives an overview of current treatments used for autoimmune diseases, as well as some future directions.

The collection of cellular and molecular basics presented in this document was prepared based on authentic and highly regarded articles and journals. The numbers in brackets indicate the references used, and all pathways have been curated for scientific knowledge and accuracy by Revvity's scientific team.

Why this guide?

This new guide continues Revvity's tradition of providing collections of specialized documents dedicated to different therapeutic areas such as immunology, neurosciences, diabetes, and NAFLD. We also give you more directly practical guides covering the expertise of Revvity's scientists in assay development and performance.

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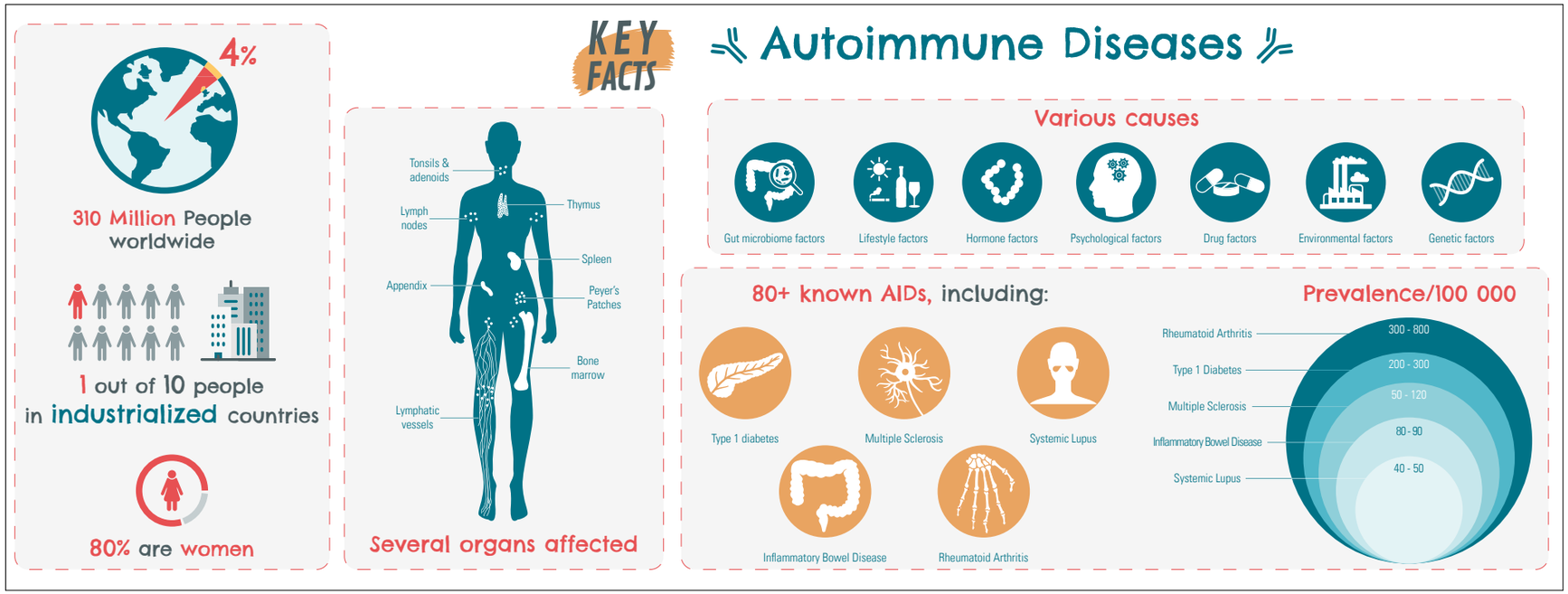
AUTOIMMUNE DISEASES AT A GLANCE

Autoimmune diseases

Like an army of experienced and disciplined soldiers, a healthy immune system fights pathogens or abnormal cells to protect the body against diseases. But if the immune system dysfunctions, it may mistakenly mount an attack against healthy cells, tissues, and ultimately organs. These autoimmune diseases can affect any part of the body, weakening organism function and even becoming life-threatening.

AIDs mainly affect women, as 80% of the patients are women, and it has been calculated that there are around 300 million patients in the world. AIDs can be triggered by genetic and/or environmental factors, so the causes vary widely. In this booklet, we focus on the main autoimmune diseases: Rheumatoid Arthritis, Inflammatory Bowel Diseases, Atherosclerosis, Lupus and Type 1 diabetes.

To date more than 80 autoimmune diseases have been reported and classified in two categories: multisystem and organ specific autoimmune diseases. Multiple Autoimmune Syndrome (MAS) refers to the combination of several autoimmune disorders. It is known that 25% of patients suffering from one autoimmune disease tend to develop additional autoimmune diseases. For example, Rheumatoid Arthritis, Systemic Lupus erythematous, Systemic Sclerosis, Sjogren's syndrome, or spondyloarthritides are multisystem autoimmune diseases. Organ-specific autoimmune diseases refer to one organ or tissue preferentially attacked by the patient's immune system. For instance, Type 1 diabetes patients suffer from pancreatic beta-cell destruction, and Vitiligo affects skin pigmentation, whereas Multiple Sclerosis affects the brain and spinal cord.



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PATHOPHYSIOLOGY OF AUTOIMMUNE DISEASES

Rheumatoid arthritis

RA is the most common chronic inflammatory disease. This autoimmune disease affects around 1% of the population in the world, and is more prevalent in females than males (1).

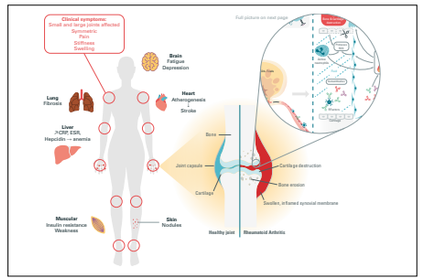
RA is a systemic and symmetric inflammatory disease associated with joint redness, hotness, stiffness, swelling and pain. Affected joints are primarily located at wrists, fingers, toes, and knees. In normal conditions, the synovium membrane surrounding the joints produces the synovial fluid which protects the joint. However, in RA inflammation of the synovium leads to an excess of synovial fluid. RA synovitis leads to cartilage damage, bone erosions, and eventually joint destruction.

Environmental and genetic factors play important roles in the disease onset. Among environmental factors, low socioeconomic conditions, diet and gastrointestinal microbiome, or smoking are the most recognized (2). Several genes have been associated with RA predisposition, such as the HLA-DRB1b gene involved in antigen presentation (3), the PTPN22 gene encoding for a protein tyrosine phosphatase involved in the negative control of T-cell receptor signal transduction, the TRAF1 gene involved in the negative regulation of signaling via TNF receptors 1 and 2 (4) and STAT4 implicated in the signaling of various cytokines like IL-12, IL-23, and type I interferons (5). In addition, epigenetic regulation of gene expression, including DNA methylation, histone phosphorylation, acetylation, has been postulated to play roles in RA pathology (6).

At the molecular level, genetic or environmental factors lead to the production of RA associated autoantigens, such as citrunillated proteins (7). Citrunillation is a post-translational modification that converts arginine into citrulline. Citrunillated proteins, such as fibrin, fibrinogen, or vimentin, are then considered to be foreign antigens and are recognized by Antigen-Presenting Cells. Autoantigen loaded-APCs migrate into the lymph nodes where they activate CD4+ helper T-Cells. In turn, these helper T-cells stimulate B-cell differentiation into plasma cells which produce autoantibodies. The two most important autoantibodies associated with RA are Rheumatoid Factor (RF) which are essentially IgM and bind to the Fc portion of IgG , and anti-citrullinated protein antibodies (ACPA) (8).Finally, CD4+ Helper T cells, plasma cells, RF, and ACPA auto antibodies migrate through the blood stream and infiltrate back into the joint.

At the joint, fibroblast-like synoviocytes constituting the synovium membrane, as well as macrophages, play a central role in the inflammation of the synovium called synovitis. Macrophages produce proinflammatory cytokines such as TNFa, IL1b, and IL6, which lead to inflammation. In addition, cytokine-activated synoviocytes secrete RANK-L and metalloproteinases. Together, cytokines and RANK-L promote the activation of the osteoclasts leading to bone erosion, whereas metalloproteinases are essentially responsible for cartilage degradation. Furthermore, infiltrated activated CD4+ T-cells secrete IL17 and RANK-L contributing to macrophage, synoviocyte, and osteoclast activation. Accentuation of bone and cartilage degradation also results from neutrophils producing proteases and reactive oxygen species (9). Moreover, synovitis associated Plasma cells participate in inflammation through cytokine and autoantibody production. Here, immune complex deposition of RF and ACPA autoantibodies with complement proteins leads to a severe inflammation. Finally, increased angiogenesis and vascular permeability reinforce immune cell infiltration into the joint.

In addition to attacks on the joints, the functionality of several organs is impaired in RA due to elevated concentrations of TNFa, IL1b, and IL6 in the circulation. The extra-articular manifestations reside i) in the liver, producing c-reactive protein and hepcidin-induced anemia, ii) in the skin, by nodule formations, iii) in the heart, by atheroma plaque formation, iv) in the central nervous system, by fatigue and depression, v) in the muscles, by insulin resistance, and vi) in the lungs, by fibrosis.



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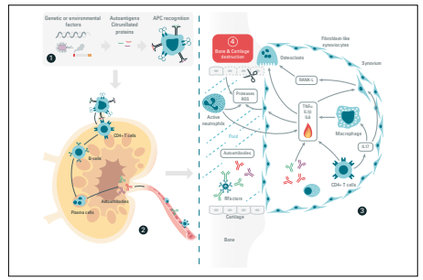

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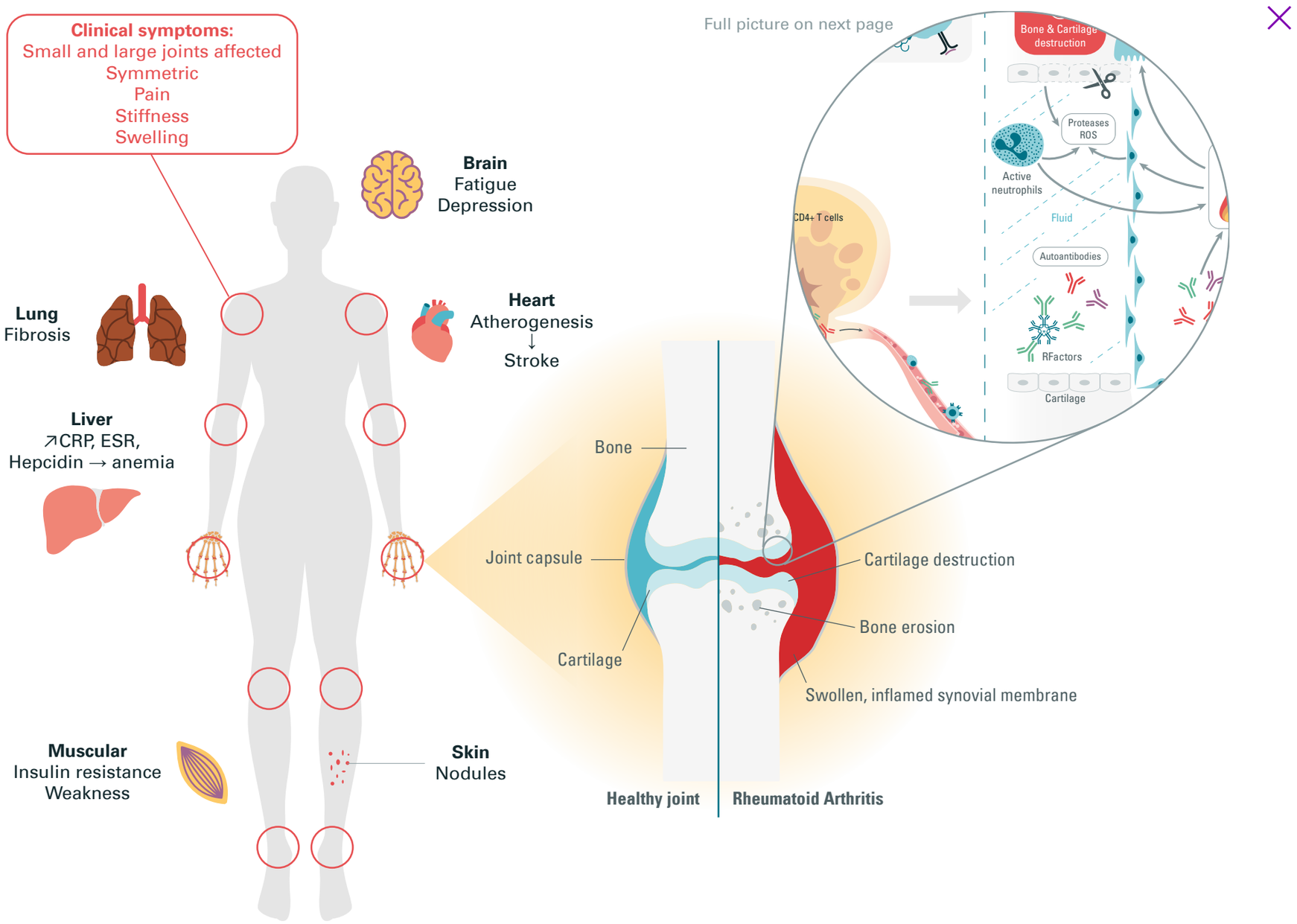

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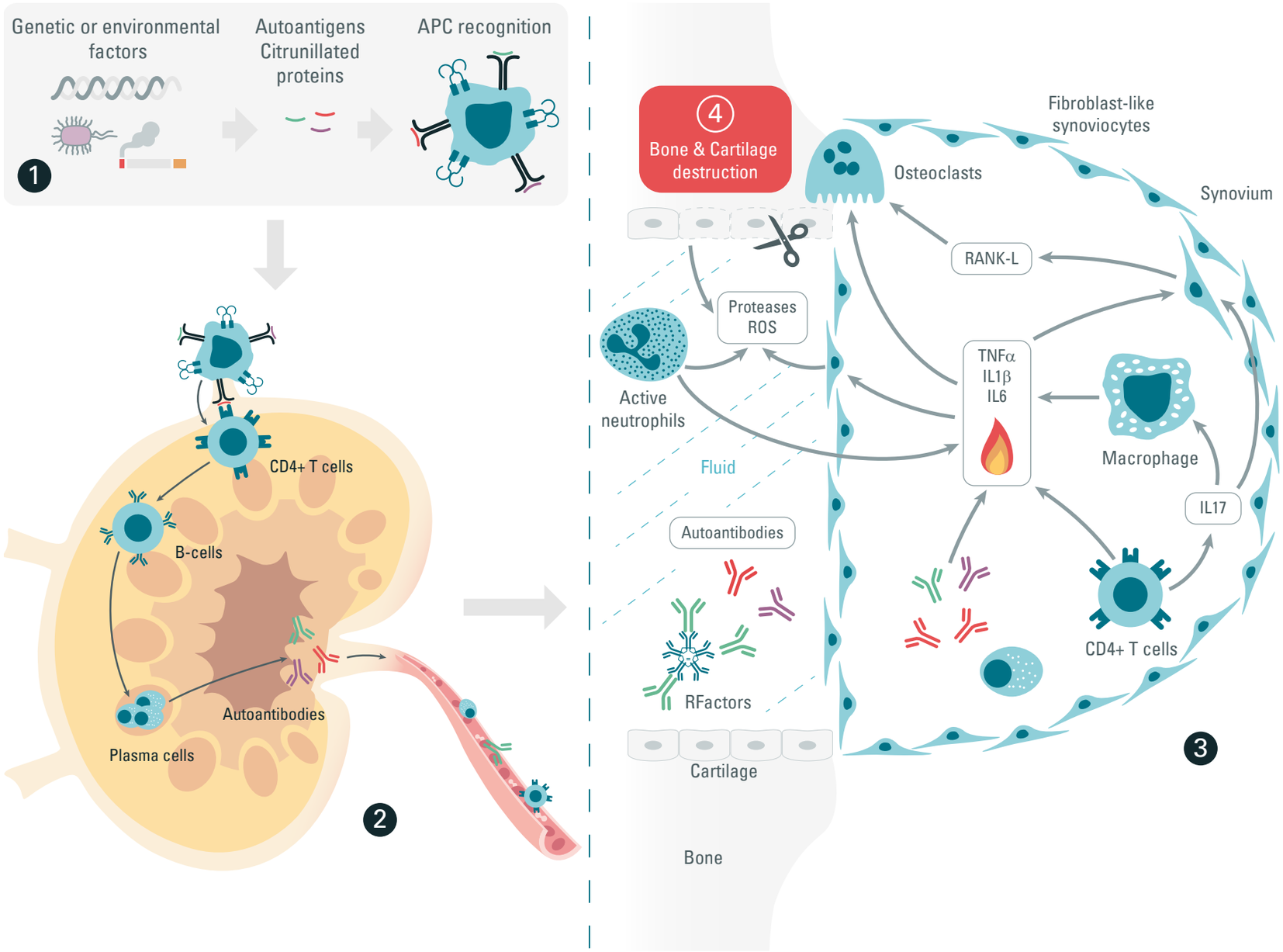

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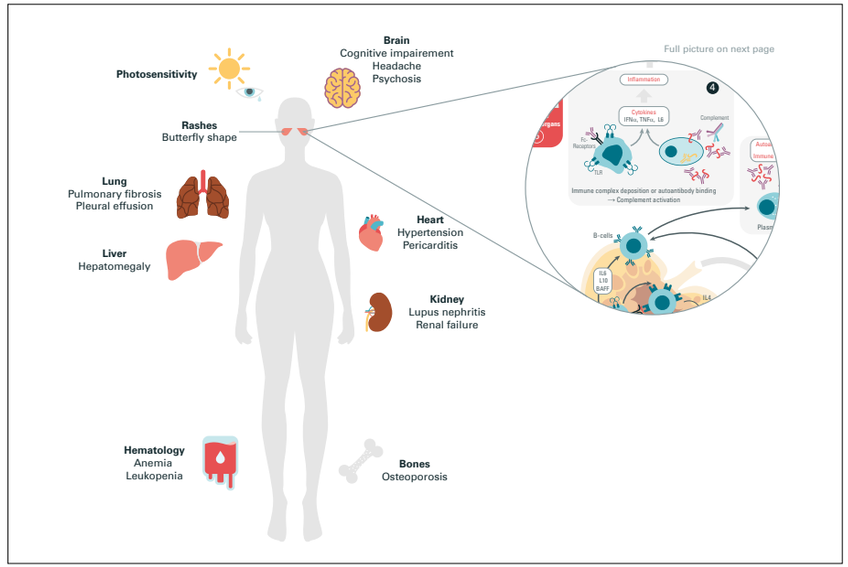
Systemic lupus erythematosus

Systemic Lupus Erythematosus, or SLE, is an autoimmune disease mediated by autoantibodies and immune complexes. SLE is a chronic multisystem disorder mainly affecting women between 15 and 40 years of age (10). Symptoms associated with SLE are fatigue, weight loss, and fever. UV sensitive butterfly rash over the nasal bridge occurs in 50% of SLE patients and represents the most common sign of SLE. Neurological affection is common and includes cognitive impairment and psychosis, as well peripheral neuropathies. Pulmonary fibrosis, vasculitis, and pleuritis are also experienced in some SLE cases, as well as heart manifestations causing chest pain and breathing issues. Lupus nephritis and ultimately renal failure result in hypertension which is a common complication of SLE. Other symptoms such as hepatomegaly, abdominal pain, nausea, chronic anemia, polyarticular arthritis, or osteoporosis are also associated with it.

SLE results from interplay between genetic, immunological, and hormonal factors, as well as environmental factors. For example, deficiencies in early complement proteins such as C1q and C4 are associated with SLE, as well as SLE-associated gene variants (e.g. IRFs, STAT4, or BLK) (11) (12). Mutations in the intracellular DNA exonuclease, TREX1, in SAMHD1 or ADAR1 genes have also been associated with lupus (13), leading to the accumulation of nucleic acids and the subsequent activation of the innate immune system. SLE has also been related to elevated Estrogen during pregnancy (14).

Environmental factors may act as initial triggers of SLE. These include UV light, viral infections such as Epstein-Barr Virus, and smoking. Environmental factors induce DNA damage and ultimately cell death through apoptosis or necrosis (15). Along with “neutrophil extracellular traps”, also called NETosis, nuclear as well as cytoplasmic and membrane components are released and exposed to the innate cells (16). Immature antigen presenting cells sense cellular material through Toll Like Receptors (TLR) or nucleic acids sensors, such as cGas or RIG-I proteins (17) (18), which in turn induce high levels of Type-I IFNs. Nuclear antigens include Histone core, dsDNA, and ribonucleoprotein complexes including SM, VI RNP, and Ro/SS-A. Cytoplasmic components also include Ro, whereas cell membrane antigens include cardiolipin found in mitochondria, platelets, or red blood cell membrane components (19).

Regardless of the antigens that are picked up by the APC, antigens are presented to naïve CD4+ T helper cells in the lymph nodes. Activated helper T-cells, mainly Th2, Th17, and follicular helper T cells, promote B cell activation, proliferation, and differentiation into plasma cells (20) (21). Then the plasma cells produce autoantibodies, such as anti-dsDNA antibodies and anti-histone antibodies, also known as ANA or Anti-Nuclear antibodies. So far more than 100 autoantibodies have been reported in SLE patients (22). These autoantibodies form immune complexes that can target virtually any organ.



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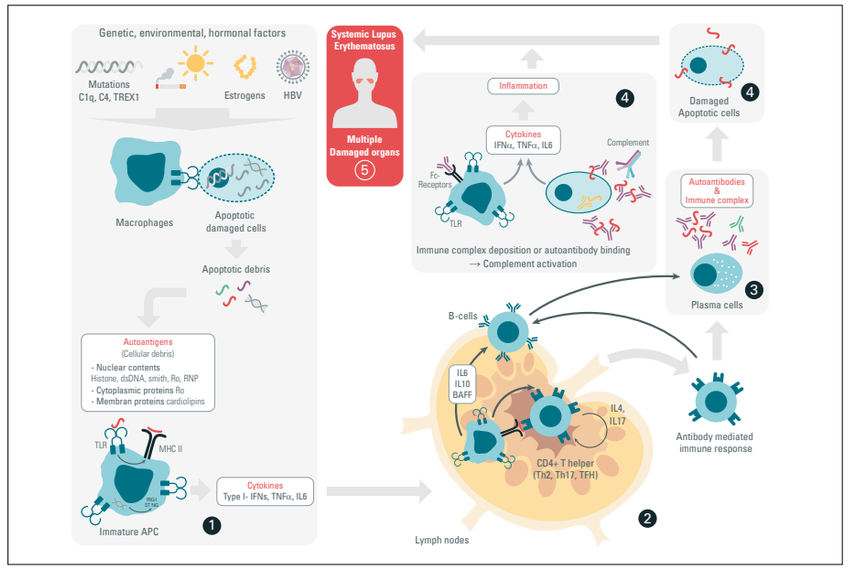
PATHOPHYSIOLOGY OF AUTOIMMUNE DISEASES

Systemic lupus erythematosus continued

Several mechanisms participate in the inflammation process which ultimately leads to tissue injury. Immune complex formation and deposition in organs results in complement activation and inflammatory response. The immune complexes can also bind to the Fc receptors of immune cells which will trigger the release of type I IFNs and proinflammatory cytokines such as TNFa or IL6, thereby increasing inflammation. The autoantibodies may bind on antigens present on the non-hematopoietic cell surface, resulting in complement activation and further cytokine releases. Finally, the sensitized immune cells detect antigens through innate immune sensors (e.g. TLR) and release proinflammatory cytokines which exacerbate the inflammation. Thus, amplified inflammation causes further organ injury, producing more damaged cells, and leading to an endless cycle.

SLE is an on-going chronic disease associated with flare-ups which reflect an immunological B cell memory and occur in response to a new exposure to antigens. Any precipitating factors causing cell apoptosis, such as sun exposure, infections, stress, or pregnancy, can trigger flare-ups and exposure to the nuclear antigens. SLE patients may have increased levels of B-cell activating factor BAFF involved in B cell survival and maturation, and antibody production by plasma cells, thus contributing to the persistence of autoreactive B cells and immunological B cell memory (23).



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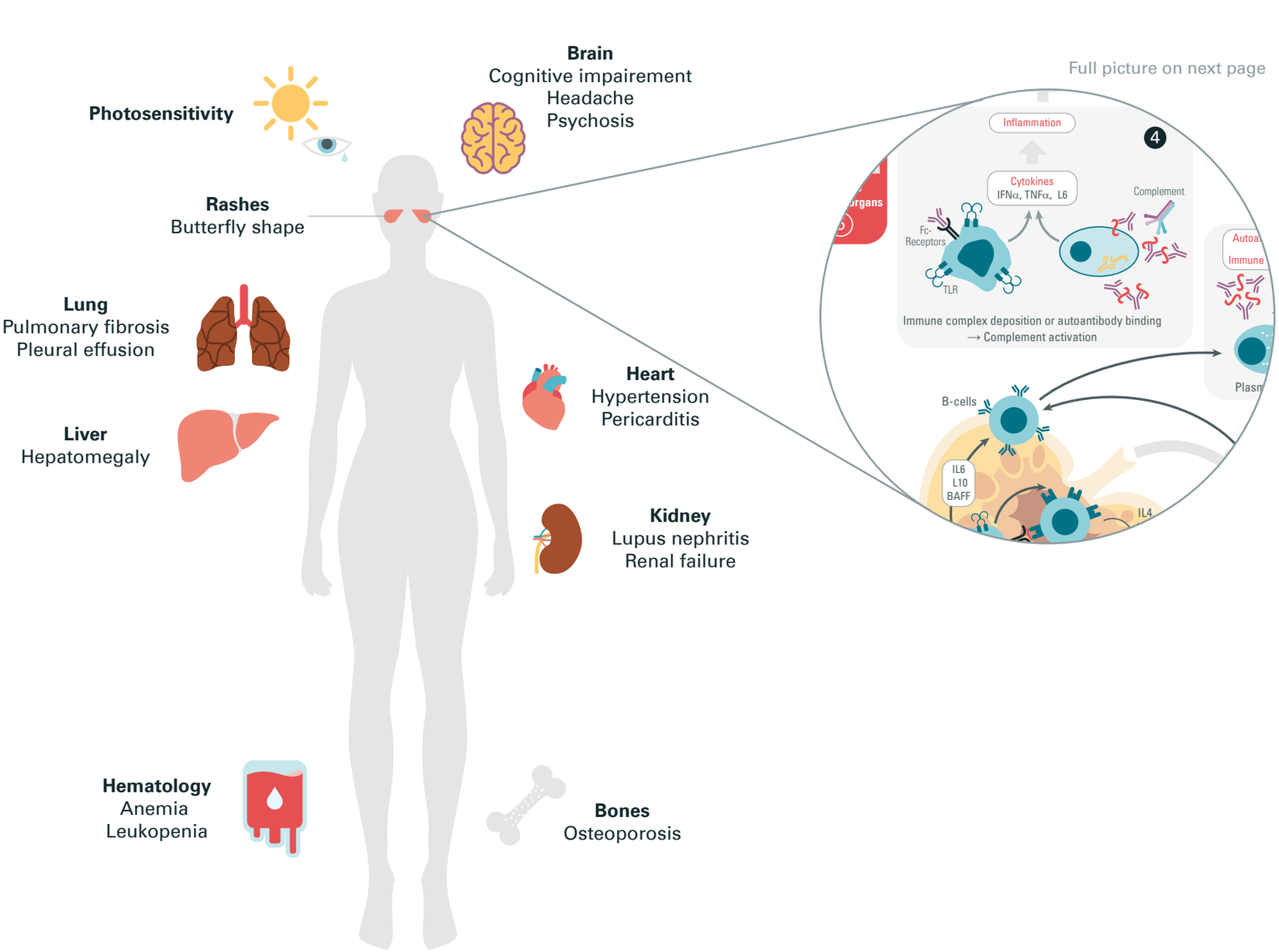

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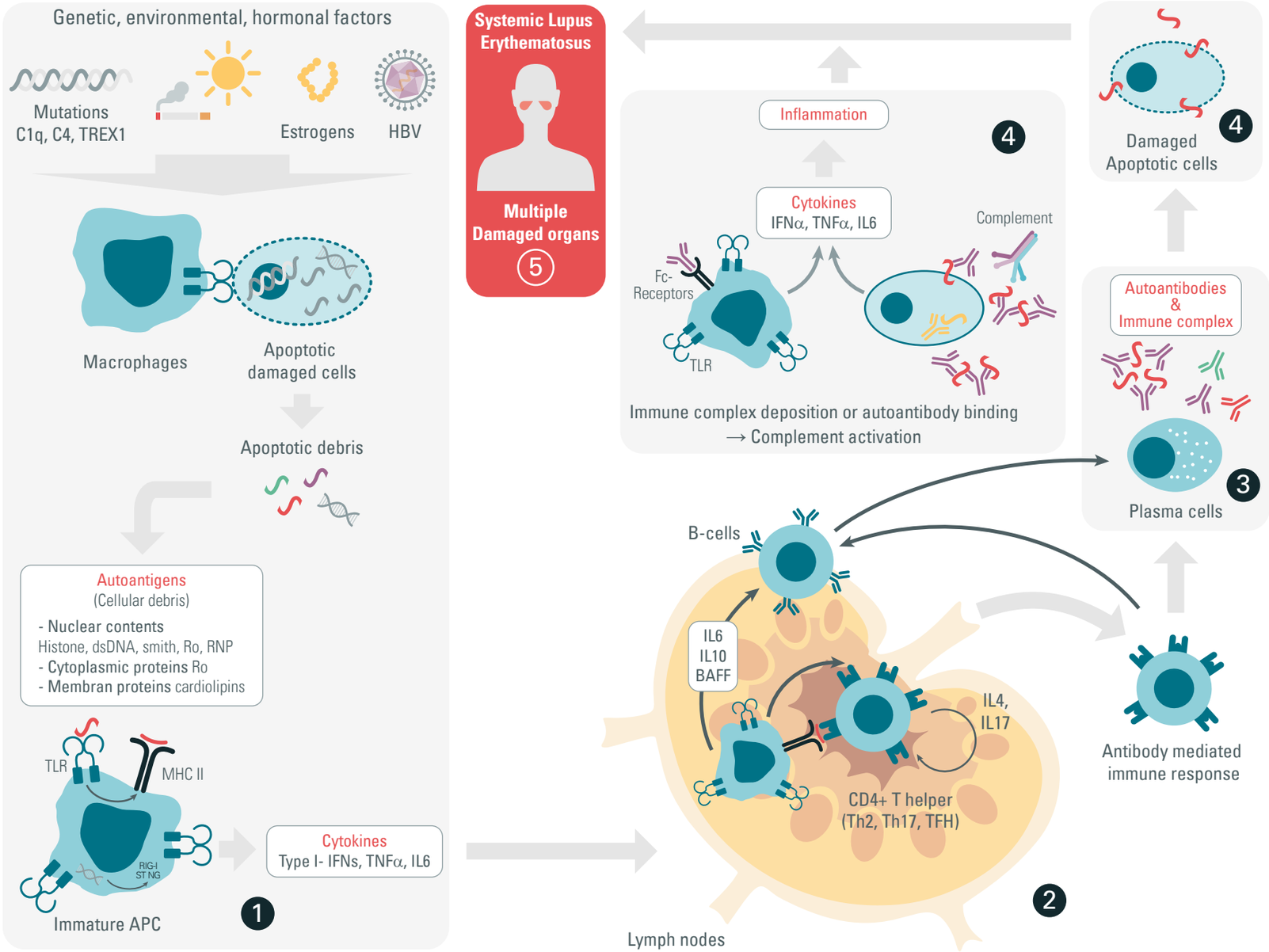

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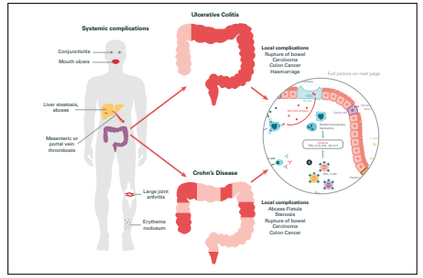
Inflammatory bowel disease

Inflammatory bowel disease (IBD) is divided into two related diseases: Crohn's Disease (CD) and ulcerative colitis (UC), and often appears in young adults between 15 and 40 years of age. UC is a continuous superficial inflammation that spreads from the distal to the proximal colon and is mainly localized in the rectal and sigmoid colon. CD is a discontinuous inflammation affecting the different cellular layers of the gastrointestinal tract, mainly the ileocecal area, the small intestine and colon. Symptoms associated with IBD can include diarrhea, rectal bleeding, abdominal pain, weight loss, and cramps. Complications associated with UC include severe bleeding, polyps, and rupture of the bowel, as well as colon cancer. CD complications include stenosis, abscess, fistula, fibrosis, and colon cancer, as well as perforation. IBD is associated with several extraintestinal manifestations, involving liver steatosis, thrombosis, joint arthritis, conjunctivitis, or mouth ulcers.

Combinations of environmental and genetic factors contribute to IBD development. For example, diet, stress, virus infections, non-steroidal anti-inflammatory drugs, or antibiotics, and smoking can promote IBD, as well as some gene variants encoding cytokines or their downstream signaling mediators (24), autophagy (e.g. ATG16L) (25), and microbial sensing (e.g. NOD2 gene) (26) (27). Dysbiosis, which refers to microbiota perturbations, has also been associated with IBD (28). In healthy patients, commensal microorganisms (e.g. B.fragilis) ensure immune protection by promoting the generation of peripheral T-reg cells and the subsequent production of the anti-inflammatory cytokine IL10. IBD patients show a high abundance of Escherichia Coli and other bacteria that promote inflammatory responses. In addition, in CD patients the mutation of the intracellular bacterial sensor NOD2 gene is associated with inflammatory gut microbiota.

Impairment of the epithelial barrier function and dysbiosis are key events in IBD initiation. Microbial peptides, which are normally translocated through M cells, pass through the lumen due to intestinal barrier impairment. Microbial and intestinal epithelial cell (IECs)-derived antigens activate APC, such as macrophages and dendritic cells, which promote CD4+ T helper cell activation. IECs, activated macrophages, and CD4+ T lymphocytes secrete proinflammatory cytokines such as TNFa, IL1b, IL18, IL17, and IL23, as well as IFNg, IL6, and IL33, resulting in chronic inflammation (29) (30) (31). In addition, antigens derived from IECs lead to B cell activation, proliferation, and differentiation into plasma cells. Autoantibodies secreted by plasma cells (Anti-Neutrophil Cytoplasmic Antibody :ANCA), along with the anti-bacterial antibodies Anti-Saccharomyces Cerevisiae Antibody (ASCA) or anti-Outer-membrane protein of Escherichia Coli (anti- OMpC) participate in the inflammatory process through the formation of immune complexes and complement activation, or the activation of macrophages through Toll-like receptors (32). Taken together, the activation of the immune system results in local and systemic complications where TNFa plays pleiotropic roles in IBD pathogenesis (33). TNFa stimulates angiogenesis, increasing the presence of immune cells locally. TNFa induces the necrosis of Paneth cells, as well as Goblet cells, halting antimicrobial functions and mucus production respectively. TNFa induces Intestinal Epithelial Cell death and impairs intestinal barrier functions. TNFa increases immune system activity, thus causing further intestinal damage. Finally, TNFa stimulates myofibroblasts to release proteases which further destroy the intestinal epithelium.



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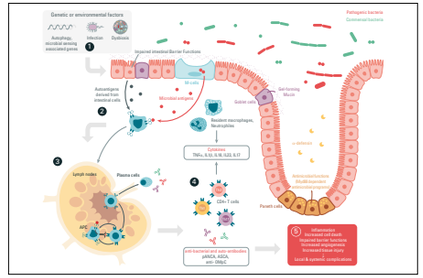

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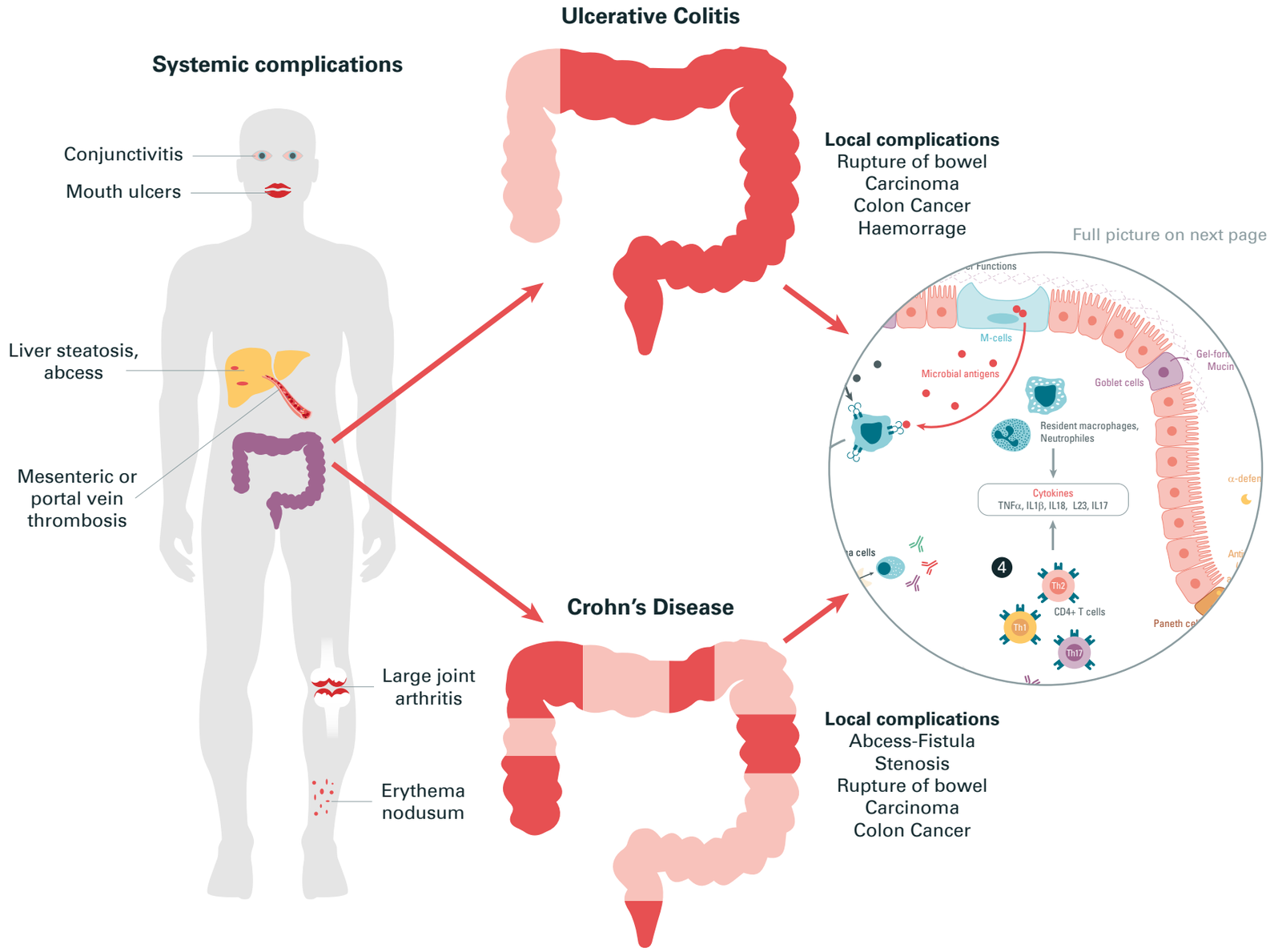

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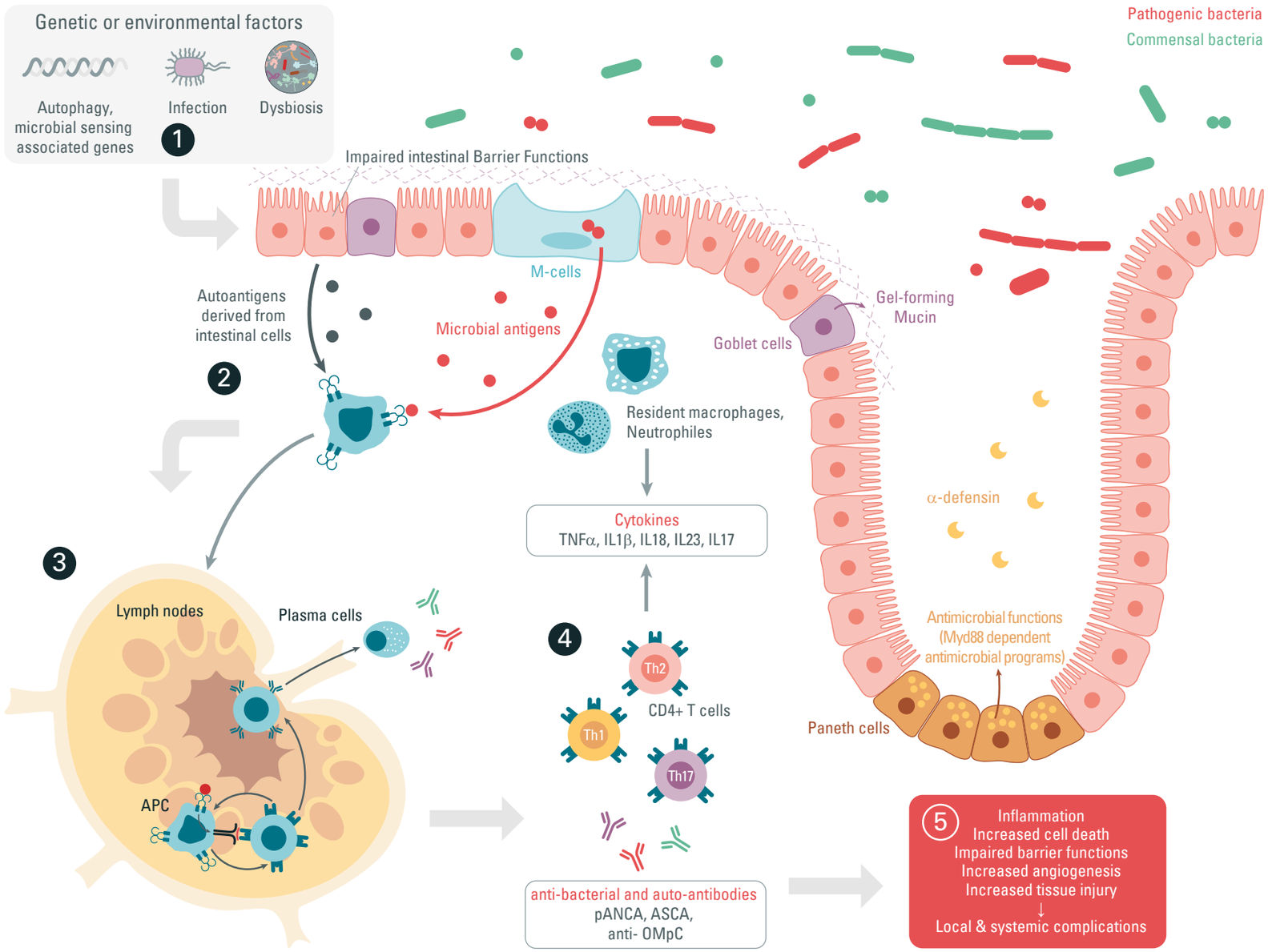

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PATHOPHYSIOLOGY OF AUTOIMMUNE DISEASES

Multiple sclerosis

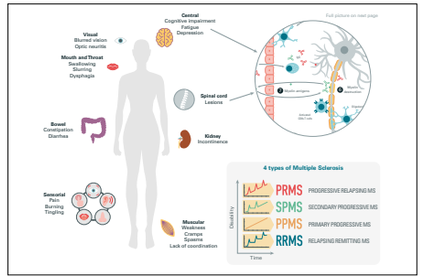
MS is a chronic organ-specific autoimmune disease targeting the Central Nervous System. MS is associated with neuroinflammation resulting in a progressive demyelination process and neuronal damage. Typical symptoms include temporary visual loss due to optic neuritis, motor and sensorial troubles, pain, fatigue, depression, neurocognitive troubles, but also impairment of bladder, bowel, and sexual functions (34). Relapsing-remitting and secondary progressive MS (RRMS, SPMS) are the two most frequent forms of MS, predominantly affecting women between 20 and 40 years of age. RRMS is marked by sequential relapses or exacerbations of symptoms followed by periods of remission, when symptoms improve or disappear. In patients suffering from SPMS, the course of the disease progressively worsens with or without periods of remission. The Primary progressive MS (PPMS) form is more resistant to treatment and symptoms become steadily worse without remissions. Progressive-relapsing MS (PRMS) is less common, progressive, and associated with flare-ups of symptoms with no periods of remission.

Despite decades of investigations, the initial trigger of MS still remains elusive. Nevertheless, the interplay between genetic, epigenetic, and environmental factors is likely to play a role in the onset and the progression of MS (35). Whereas the involvement of epigenetically regulated allele variant HLA DRB*1501 has recently been found in MS pathogenesis (36) (37), the HLA-A2 allele seems to be protective. Viral infections, particularly with herpes virus, low Vitamin D due to reduced sun exposure, diet and obesity, gut microbiota, air pollution, but also smoking have been identified as contributing to MS (38) (39).

The perturbation of peripheral immune responses, as well as brain cells such as microglia or astrocytes, convey an autoimmune process to the CNS.

Whether the antigens inducing immune activation are molecular mimicry (viral or bacterial peptides that share homology with brain proteins such as myelin) or myelin antigens (40), activated CD4+ Th1, CD4+ Th17, and CD8+ peripheral T cells infiltrate the CNS where they secrete pro-inflammatory cytokines such as IL2, IFNg, TNFa, or IL6. CD8+ cytotoxic T cells directly attack the oligodendrocytes and the myelin, promoting their destruction (41). Activated microglia, macrophages, NK cells, or neutrophils participate in pro-inflammatory cytokine secretion and thereby contribute to neuronal damage and a demyelination process. In addition, activated B cells secrete autoantibodies which amplify the CNS attack, through complement activation or macrophages inducing neuron damage (41). Of note, even though 90% of the patients have elevated levels of CSF IgG, only a few specific autoantibodies have been attributed to MS, with Anti-myelin oligodendrocyte glycoprotein (MOG) being one them (42) (43) (44). Furthermore, a continuous Myelin antigen release induces myelin autoantibody production and further amplifies the demyelinating circle. Finally, impaired astrocytes and blood brain barrier functions contribute to disease progression.

For more information regarding neuroinflammation and neurodegenerative diseases, please read our Neuroscience guide.



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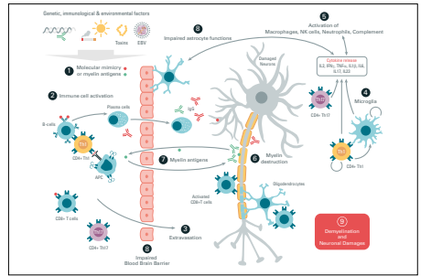

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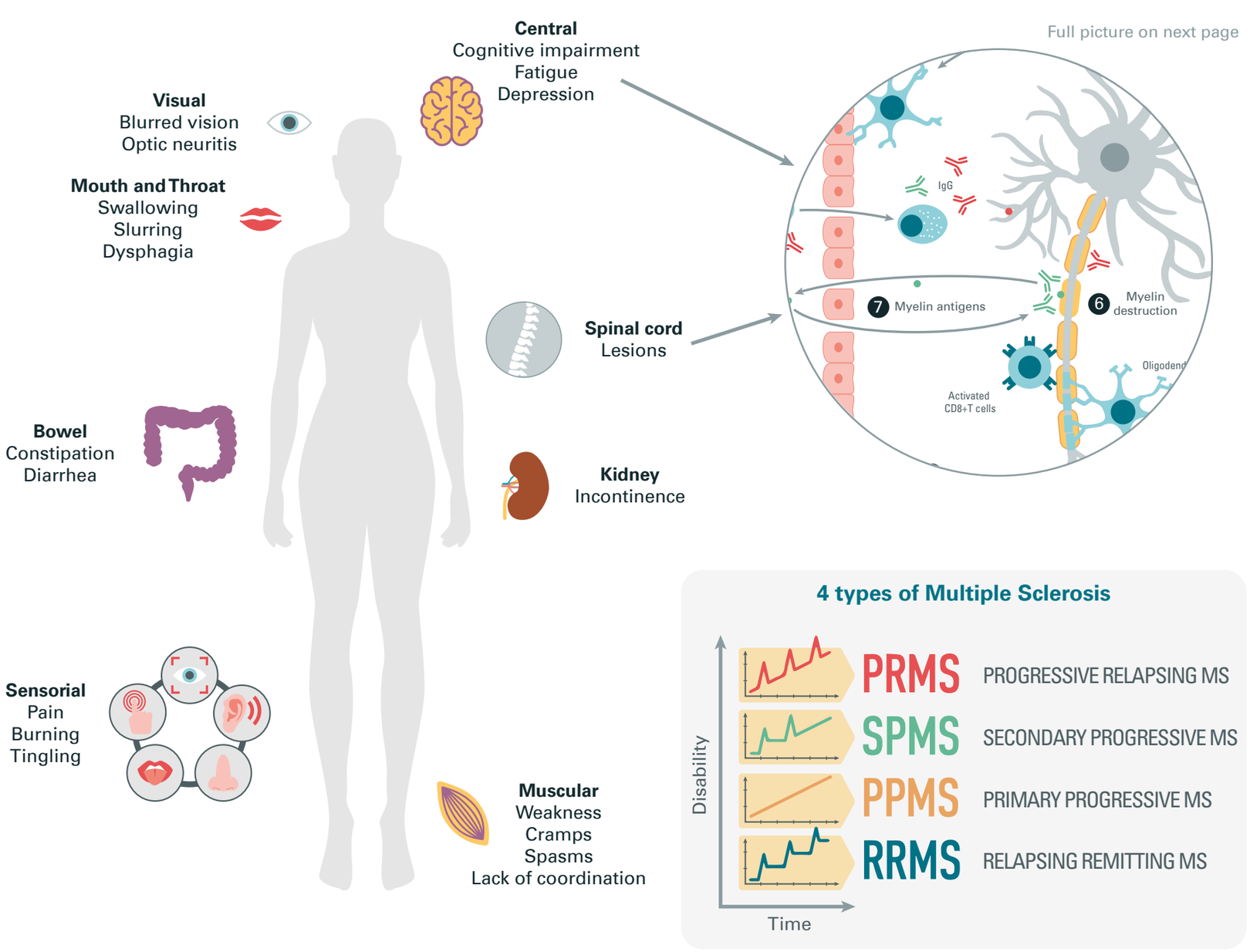

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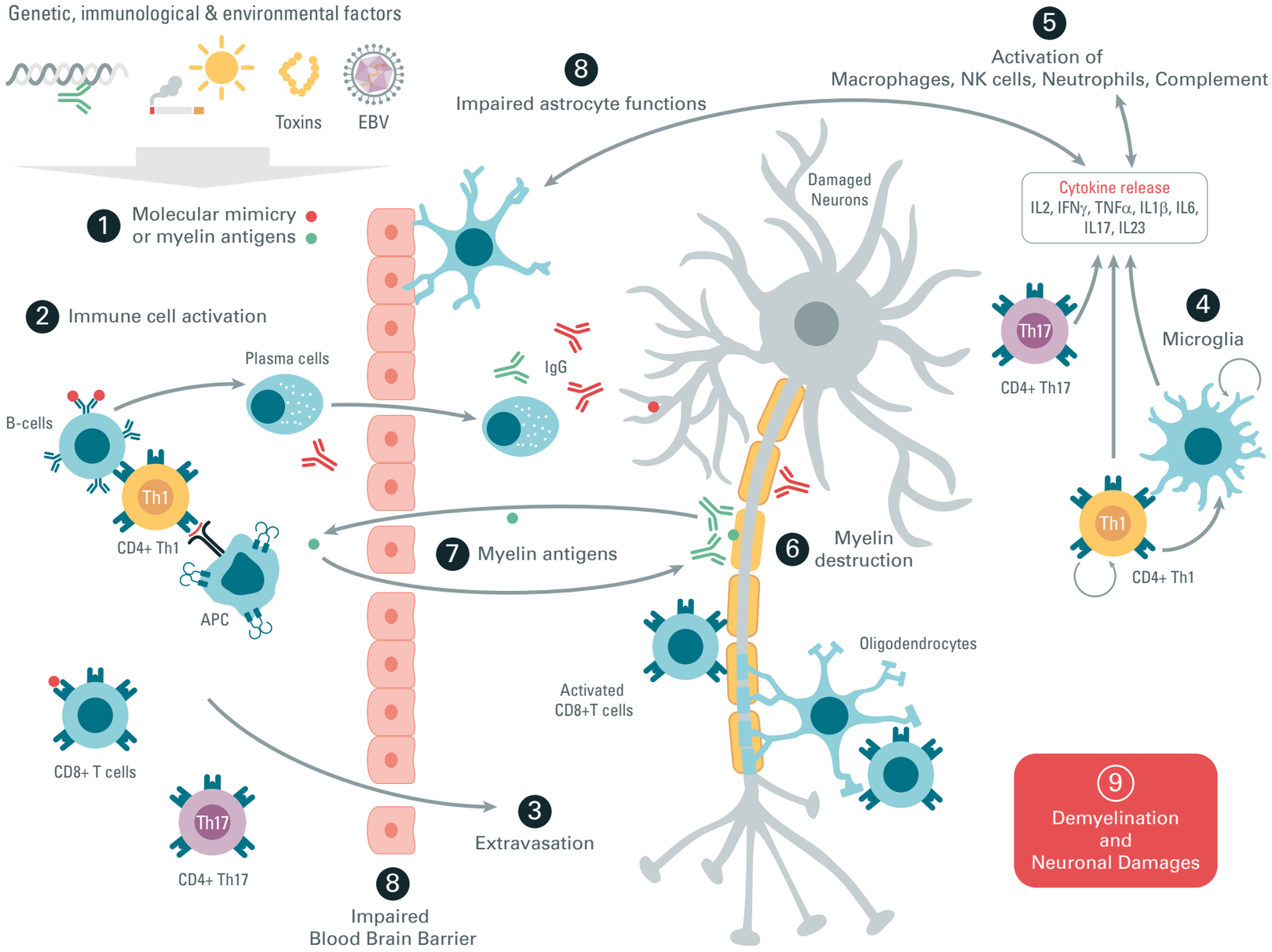

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PATHOPHYSIOLOGY OF AUTOIMMUNE DISEASES

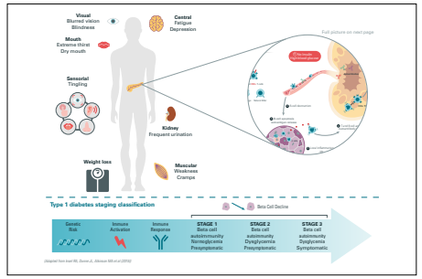
Type 1 diabetes

Type 1 diabetes is a chronic autoimmune disease, less common than its sibling Type 2 diabetes. Autoimmune diabetes represents 10% of diabetes patients and is the most common form affecting children under 15 years of age. Mellitus diabetes is characterized by high blood glucose levels due to pancreatic islet beta-cell destruction and insulin deficiency.

The pathogenesis of autoimmune type 1 diabetes is divided into three stages (45). Stage 1 is presymptomatic, the blood glucose level being normal (normoglycemia), and relates to β-cell autoimmunity with the presence of islet autoantibodies. Stage 2 is still presymptomatic, but glycemia is perturbed, and finally stage 3 sees the onset of symptomatic disease. Typical symptoms of diabetes include frequent urination, increased thirst, hunger but weight loss, fatigue and irritability, but also blurred vision and skin infections. Left untreated, type 1 diabetes can lead to life-threatening complications through diabetic ketoacidosis (DKA) such as neuropathy, nephropathy, heart and blood vessel disease, and coma.

Even though the initial triggers of T1D pathogenesis remain to be elucidated, the interplay between genetic factors, mainly HLA alleles (46), central and peripheral tolerance, as well as environmental factors contribute to the disease onset. Autoreactive T cells, neutrophils, and NK cells as well as macrophages infiltrate the beta-pancreatic islets, leading to inflammatory cytokine production such as TNFa, IL1b, and IFNg, and local inflammation. Pancreatic insulin-producing β cells undergo apoptosis, liberating autoantigens which are then recognized by Antigen Presenting Cells and migrate to the lymph nodes. Finally, activated CD4+ and CD8+ T cells, as well as autoantibody-producing differentiated B cells leave the lymph node and migrate back to the pancreas, where they exert their cytotoxic activities, leading to β-cell destruction and T1D (47) . Major islet autoantibodies target insulin (IAA), GADA, islet antigen-2 (IA-2A), or the zinc transporter 8 (ZnT8A) (48).

For more information regarding diabetes and associated signaling pathways, please read our Diabetes guide.



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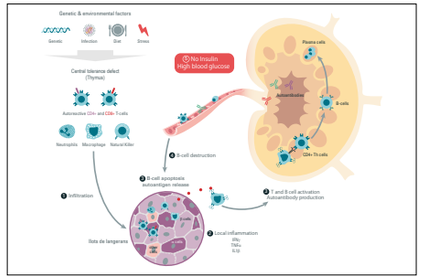

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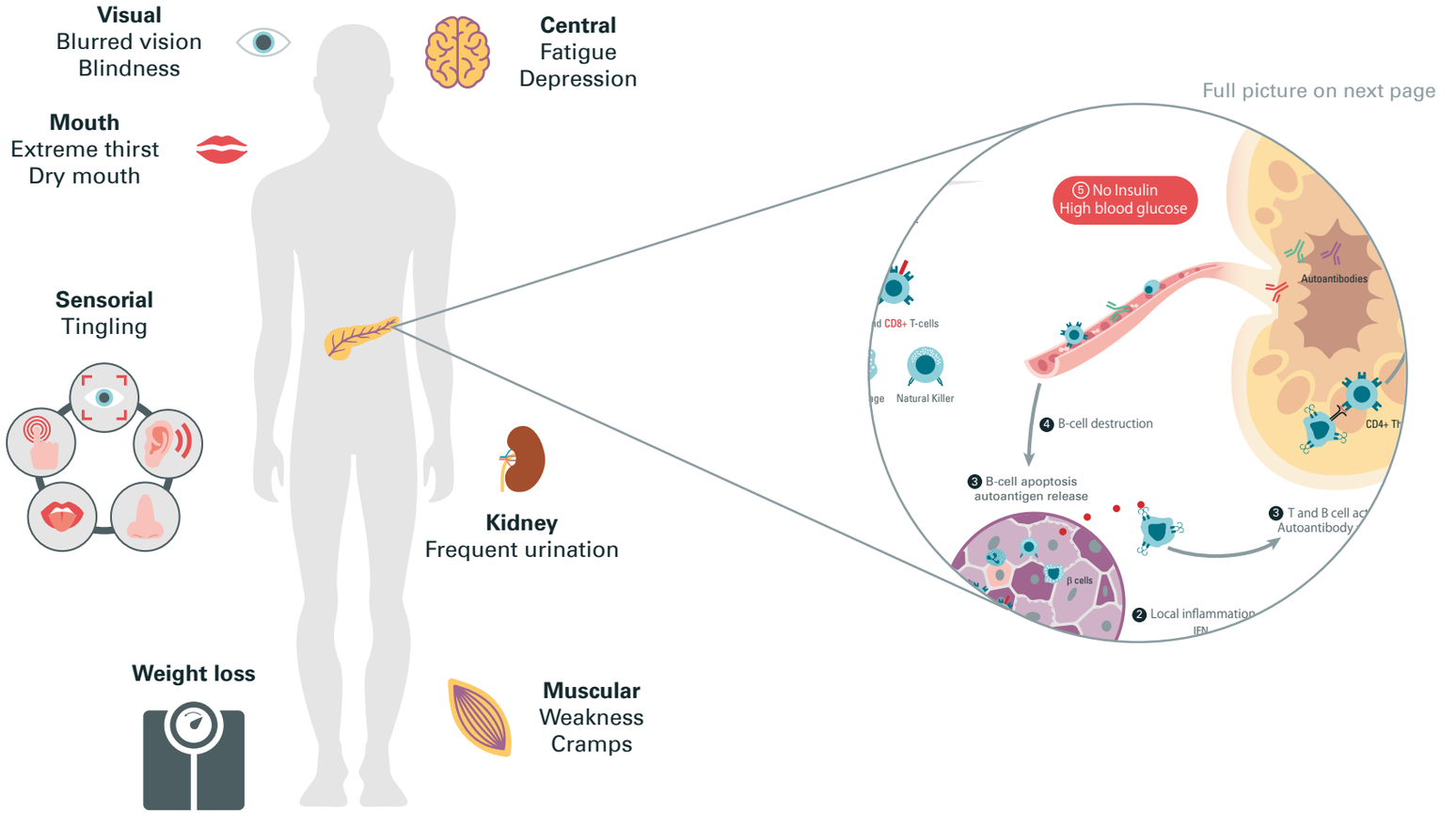
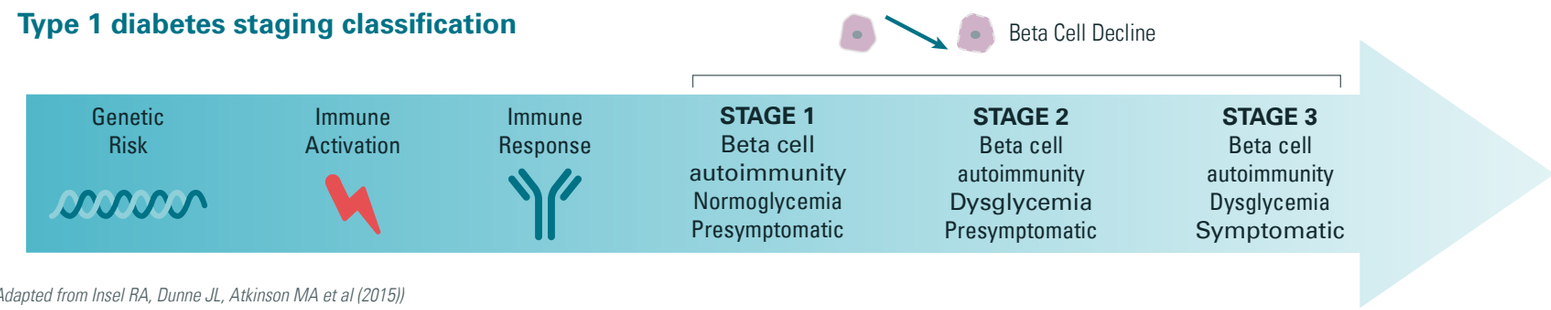


Type 1 diabetes staging classification



(Adapted from Insel RA, Dunne JL, Atkinson MA et al (2015))

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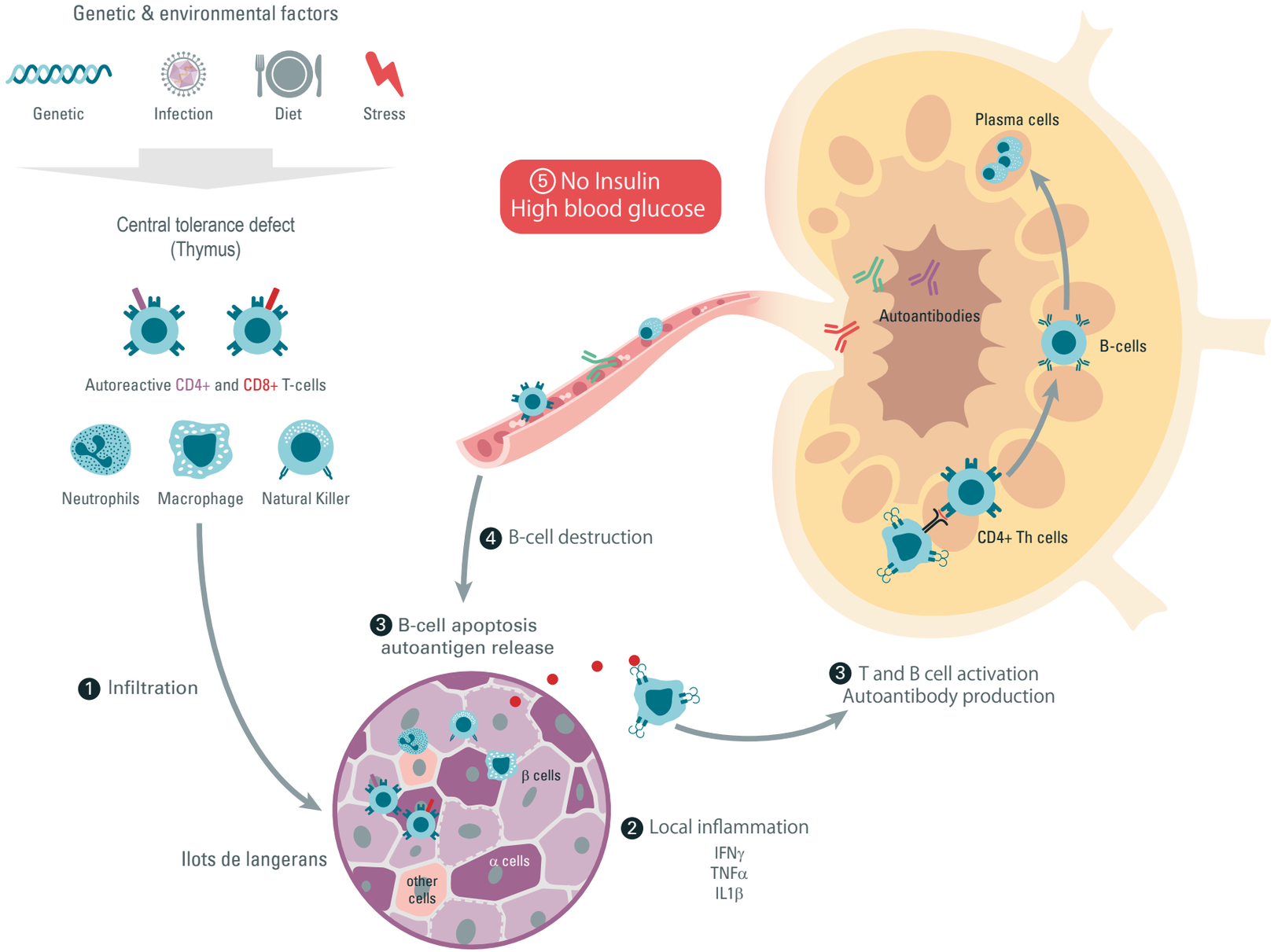

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Common antigens and autoantibodies associated with AI

At some point, autoimmune diseases are elicited by antigens, whether self or microbial antigens, so-called molecular mimicry. These antigens can be captured by Antigen Presenting Cells, leading to the production of autoantibodies which in turn result in harmful effects in tissues.

The main autoantibodies associated with the previously described autoimmune diseases, as well as their targeted components, are listed in the following table:

AUTOIMMUNE DISEASES	AUTOANTIBODIES	TARGETED COMPONENTS
Rheumatoid Arthitis	Rheumatoid factor (RF)	Fc portion of IgG.
	Anti Citrullinated Protein Antibodies (ACPA)	Fibrinogen, vimentin, filaggrin, CII, enolase.
	anti-RA33	hnRNP
Systemic Lupus Erythematosus	Anti Nuclear antibody	Nuclear components
	Anti-dsDNA	Double stranded DNA
	Anti-Sm	snRNPs complex
	Anti-Ro/SSA	hY-RNP complex
	Anti-La/SSB	Calmodulin-Binding Protein
	Anti-Cardiolipin	Mitochondrial protein
Inflammatory Bowel Diseases	perinuclear AntiNeutrophil Cytoplasmic Antibodies	Histone H1
Multiple Sclerosis	Anti-MOG	Myelin Oligodendrocyte Glycoprotein (MOG)
	Anti-AQP4	Aquaporin-4 (water channel protein)
Type 1 Diabetes	Anti-IAA	Insulin
	Anti-GADA	Glutamic Acid Decarboxylase
	Anti-IA-2A	Islet antigen-2
	Anti-ZNT8A	Zinc transporter 8

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CELLULAR AND MOLECULAR MECHANISMS OF AI DISEASES

Central and peripheral tolerance

In a healthy condition, our immune system does not recognize self-antigens and cannot mount an attack against our own tissues. This is known as "self-tolerance", encompassing immune central and peripheral tolerance. The central tolerance mechanism occurs in lymphoid organs: the thymus for T-cells and the bone marrow for B-cells, where T or B lymphocytes that recognize self-antigens are eliminated or inactivated. Peripheral tolerance occurs in peripheral lymphoid organs, such as lymph nodes and spleen.

CENTRAL TOLERANCE

T-lymphocytes, produced in the bone marrow, are matured in the thymus, where different spatial and temporal steps orchestrate the differentiation and selection process (49) (50) (51) (52) (53).

In the capsular region, double negative T-cells (DN) express mainly TCRab but not the CD4 or CD8 co-receptors, and undergo TCR gene rearrangement, which provides the diversity of the TCR repertoire.

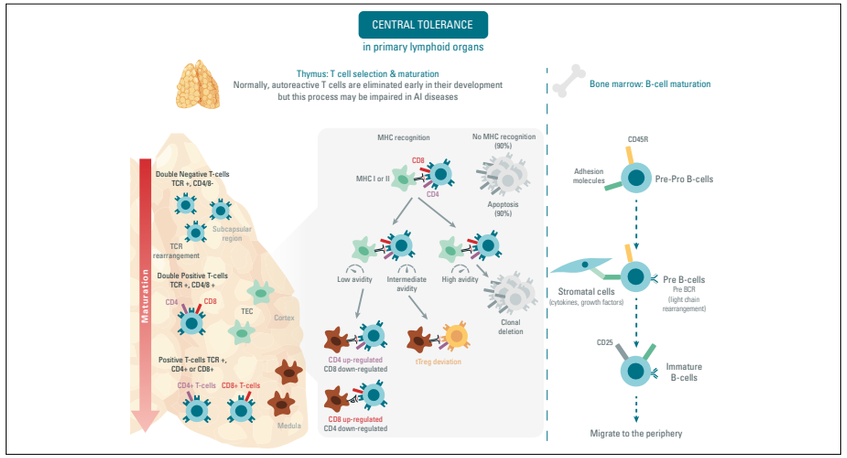
Then the DN cells move down to the cortex where they express both CD4 and CD8 co-receptors, now being called double positive T-cells (DP). Here DP interact and recognize a large panel of self-antigen peptides displayed on MHC-I or II expressed at the cell surface of thymic epithelial cells (TEC). This can be considered as the first degree of T-cell education and editing. Different selection procedures occur:

- A positive selection ensures T-cells strictly recognize peptide bound MHC; otherwise they undergo cell death, which occurs for 90% of T-cells.
- A negative selection ensures T-cells do not recognize self-antigens. Here the remaining 10% of T-cells displaying high avidity for MHC bound peptides are eliminated by apoptosis. In many autoimmune diseases, this negative selection is defective, which leads to the production of autoreactive T cells.

Moving further down in the medulla, the DP T-cells recognize either MHC class I or MHC class II present on mTEC. When recognizing MHC-II, CD4 expression is up-regulated whereas CD8 is down-regulated, giving birth to CD4+ T helper Lymphocytes. Conversely, when recognizing MHC-I, CD8 expression is up-regulated whereas CD4 is down-regulated, giving birth to CD8+ cytotoxic T-cells. In addition, agonist selection is a process enabling the deviation of intermediate avidity self-reactive CD4+ T-cells into thymic Tregs.

Thymic education is then completed, and T cells spread through our body, essentially in the peripheral lymph nodes where they encounter dendritic cells or other Antigen-Presenting Cells and continue their maturation and expansion.

B-cells' primary development occurs in the bone marrow where hematopoietic stem cells successively differentiate into Pro B-cells, Pre-B cells, and finally immature (also called naïve) B-cells which carry a unique membrane-bound form of antibody constituting the antigen receptor or BCR. Immature B-cells move to the periphery e.g. spleen or lymph nodes for further maturation, activation, or differentiation into plasma cells.



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CELLULAR AND MOLECULAR MECHANISMS OF AI DISEASES

Central and peripheral tolerance

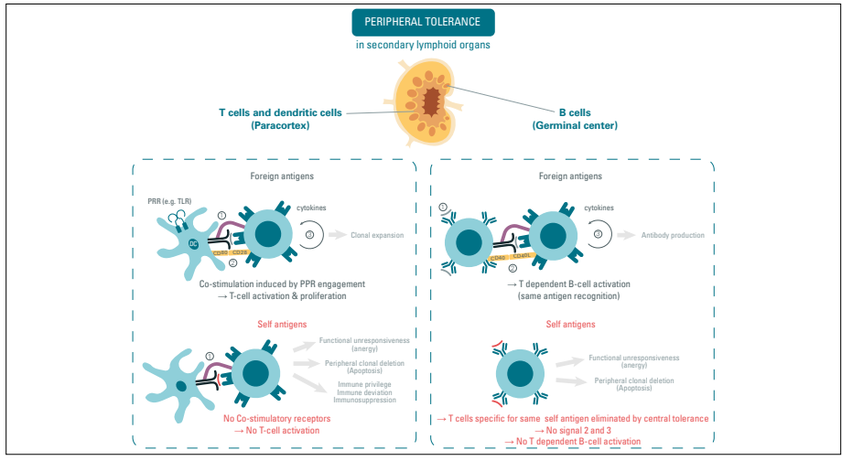
Central B-cell tolerance is controlled by multiple mechanisms at different B-cell differentiation stages, preventing the generation of auto-reactive B-cells and the subsequent autoantibody production involved in autoimmune diseases. In the bone marrow, Immature B cells encounter a wide variety of multivalent and monovalent self-antigens which are displayed by stromal cells, hematopoietic cells, and circulating molecules. If BCR from immature B cells do not, or only weakly, interact with multivalent self-antigens, they successfully leave the bone marrow and migrate to the peripheral lymphoid organs. Conversely, if the interaction between BCR and self-antigens is strong, further development of the potential auto-reactive B-cells is arrested. Three mechanisms are involved in acquiring central B-cell tolerance: receptor editing, clonal deletion, and anergy. Receptor editing is a process by which V and J genes encoding the BCR light chain undergo a new rearrangement. Receptor editing is the main process contributing to the elimination of autoreactive B cells. If receptor editing is not successful, then apoptosis of autoreactive B-cells occurs, which is known as clonal deletion. If BCR interact with monovalent soluble self-antigens, autoreactive immature B cells are functionally inactivated by a process called anergy. These anergic immature autoreactive B cells leave the bone marrow and migrate to the peripheral lymphoid organs, where they eventually undergo apoptosis. (54) (55) (56)

PERIPHERAL TOLERANCE

When central tolerance mechanisms are not efficient, self-reactive T and B cells escape and migrate to the peripheral lymphoid organs, where several peripheral tolerance mechanisms efficiently keep them in check. In fact, naïve T cells exhibit high levels of CCL21 receptor (CCR7) as well as receptors for adhesion molecules, and can only migrate to secondary lymphoid organs which specifically express CCL21 chemokine and adhesion molecules. Thus, naïve autoreactive T cells are excluded from peripheral tissues. In addition, some organs such as brain, eyes or testis are immune privileged areas where the immune cell numbers are intrinsically low (57).

Unlike DCs presenting foreign antigens, DCs presenting self-antigens express low levels of CD80 or CD86 co-stimulatory receptors which are necessary for complete T cell activation (58) (59). Thus, autoreactive T-cells recognizing MHC-II presenting self-antigens cannot undergo activation but instead remain inactivated through anergy (60). Anergy, which refers to a long-term functional unresponsiveness state, can also result from the activation of inhibitory receptors such as CTLA-4, PD-1, and Lag3 present on the T-cell and which inhibit TCR signaling (61). Self-reactive T cells can undergo apoptosis through Activation Induced Cell Death (AICD) via Fas-Fas Ligand signaling and resulting in clonal deletion (62). Whereas clonal deletion and anergy prevent autoimmune responses, other mechanisms control or dampen autoimmune reactions. Among these peripheral tolerance mechanisms, Tregs are key players (see Regulatory T-cells in self-tolerance).

Autoreactive B cells which bind self-antigens in the periphery require co-stimulatory signals from helper T-cells (through CD40-CD40L and cytokines), which in such cases would be autoreactive T-cells. Thanks to the peripheral mechanisms cited above, autoreactive T-cells are eliminated. Thus, autoreactive B-cells which cannot receive activation signals from T-cells become anergic, and eventually die by apoptosis.



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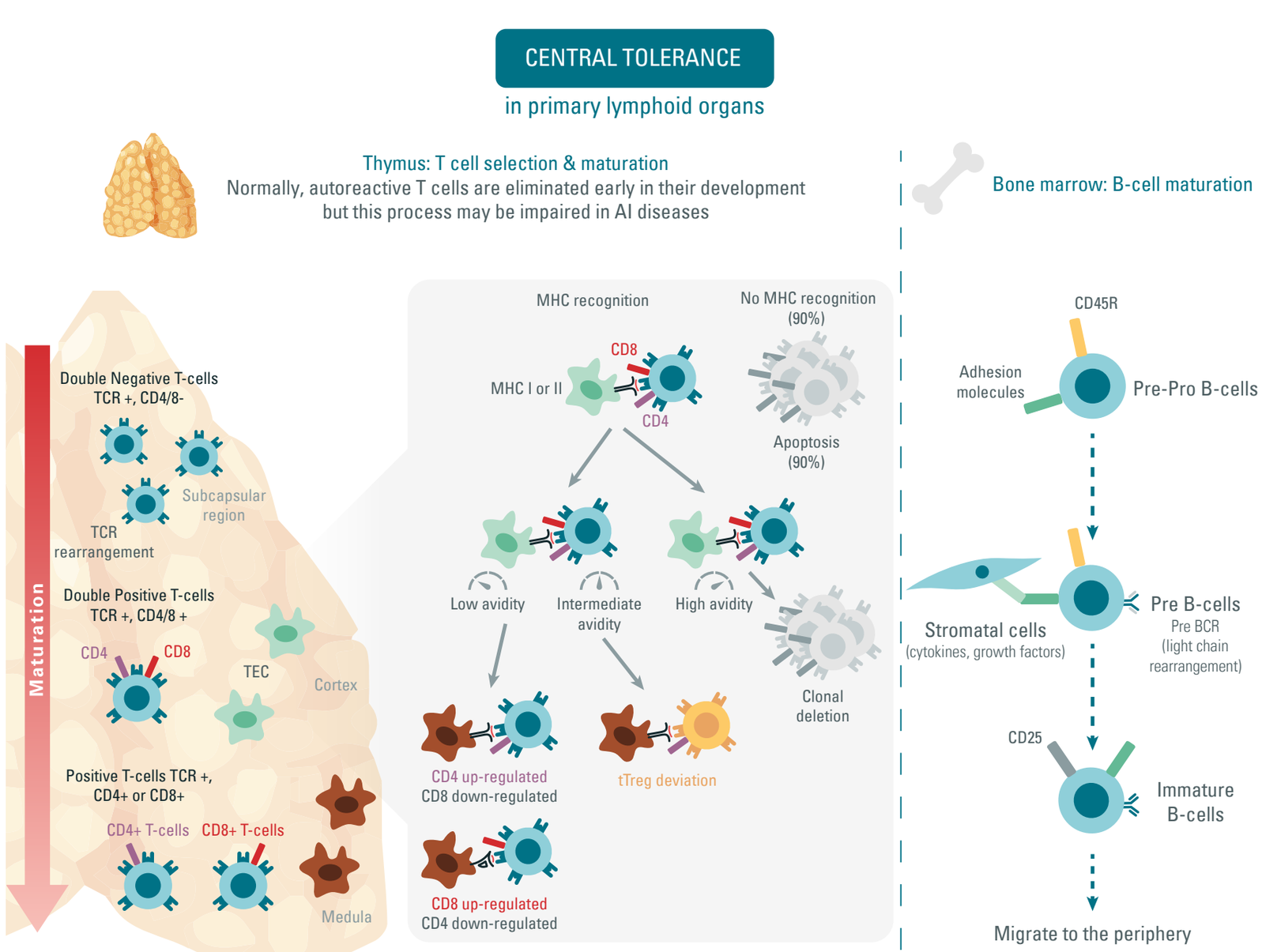

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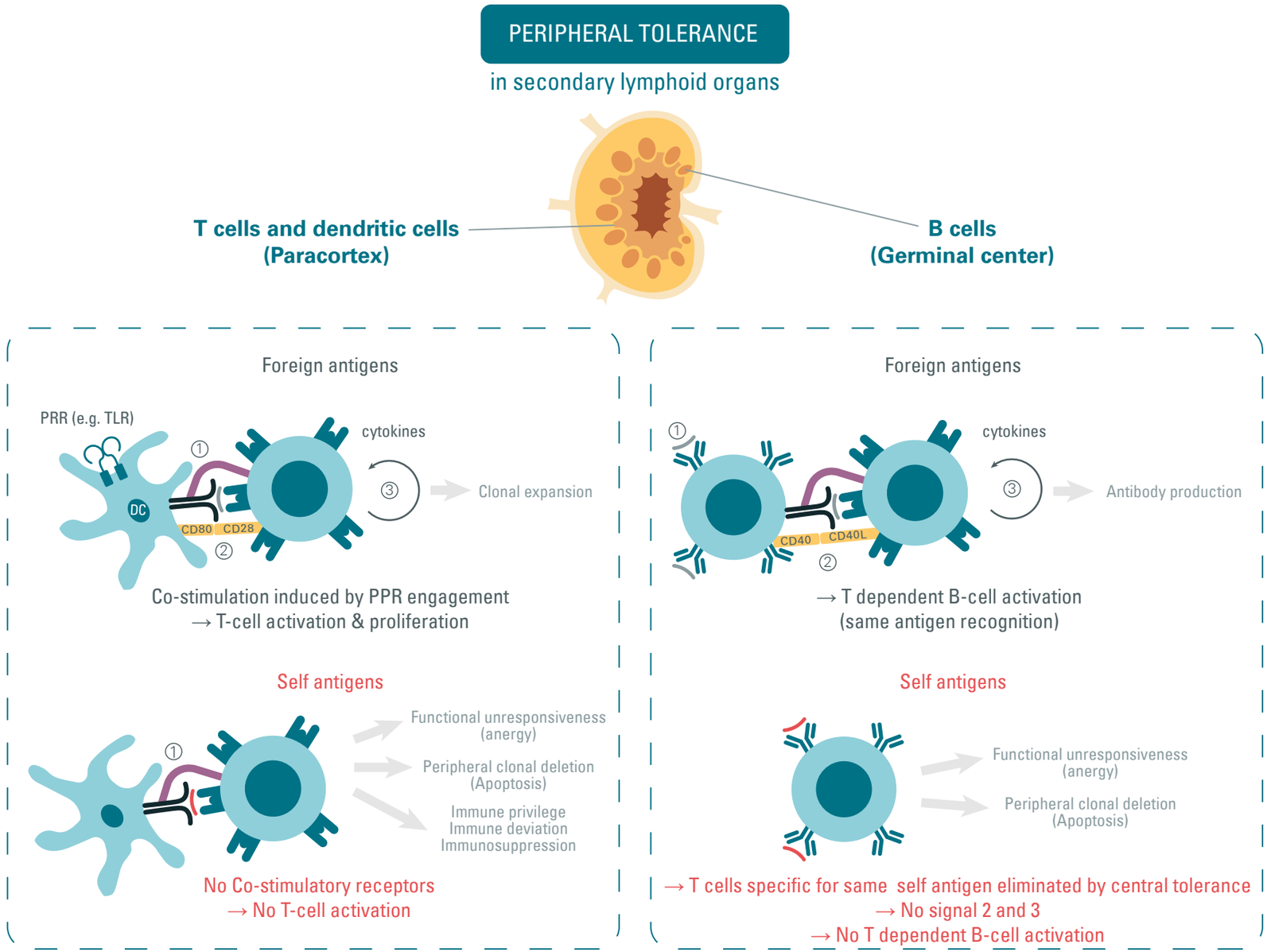

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CELLULAR AND MOLECULAR MECHANISMS OF AI DISEASES

Central and peripheral tolerance

REGULATORY T-CELLS IN SELF TOLERANCE

If Thymic Tregs are involved in central T cell tolerance, peripheral Treg cells play essential roles in the immunosuppressive mechanisms leading to peripheral tolerance.

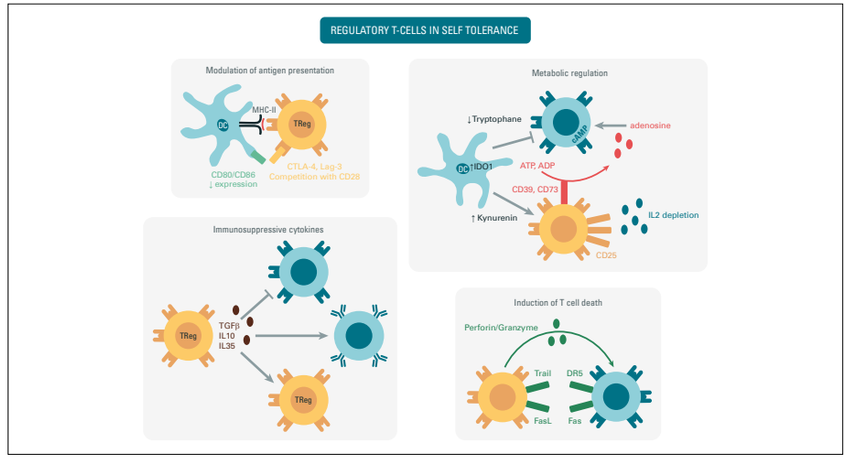
Direct and indirect immunosuppressive mechanisms exerted by Treg include:

- **Modulation of antigen presentation,** which is a key mechanism for Treg mediating suppression of effector T cells (63) (64). CTLA-4 is an immune checkpoint inhibitor constitutively expressed on the surface of Treg, which i) competes with CD28 expressed by effector T-cells for binding to CD80/86 ligands on DCs, and ii) down-regulates CD80/86 expression on DCs. Other immune checkpoint inhibitors like Lag-3 are also involved in this mechanism.
- **Metabolic regulation.** Tregs can induce the expression of Indoleamine 2,3-dioxygenase by DCs. Enzymatic activity of IDO catalyzes the production of kynurenine from Tryptophan, which promotes Treg differentiation through aryl hydrocarbon receptor (65) (66). In addition, Tregs express the CD39 and CD73 ectonucleotidases, which produce Adenosine from ATP or ADP. Adenosine binds the A2A receptor on effector T cells, which in turn results in their direct suppression due to increased levels of cAMP (67) (68).
- **Production of immunosuppressive cytokines.** Tregs can secrete TGFβ, IL10, or IL35, which inhibit effector T cells and DCs (69) (70) (71). In addition, Tregs express a high CD25 level which has high affinity for IL-2. Therefore, by consuming and depleting IL-2, Tregs prevent effector T cell activation (72).
- **Induction of effector T cell cytolysis.** Tregs can secrete Perforin, Granzyme A, and Granzyme B, which lead to effector T cells (73) (74). Apoptosis of Effector T cells induced by Tregs can be mediated by other molecules, such as TRAIL-DR5, FasL-Fas, Galectin-9-Tim3, and Gal-1 (75).

In addition to their role on effector T cells, Tregs are also involved in the suppression of autoantibody production by B cells (76).

Breakdown of central or peripheral tolerance mechanisms represents one cause of autoimmune disease onset.

It has been found that MHC gene variants are the most frequent genes associated with autoimmune diseases. Other genes related to immune defects, such as PTPN22 (protein phosphatase), CTLA4 (Checkpoint inhibitor), IL23R and TYK2, AIRE (transcription factor involved in central tolerance), FOXP3 (transcription factor involved in T-Reg lineage), IFIH1, DNASE1, TREX1, C1Q, or C4A (complement factors), have also been identified in certain autoimmune diseases.



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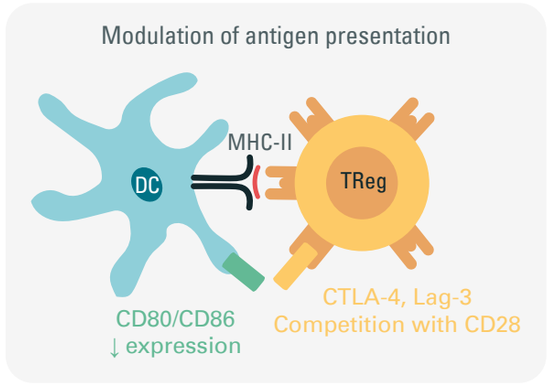
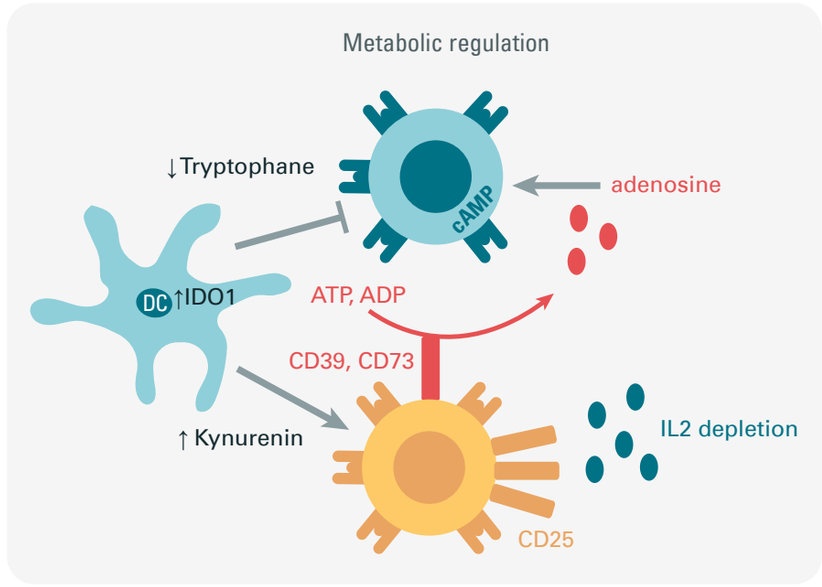
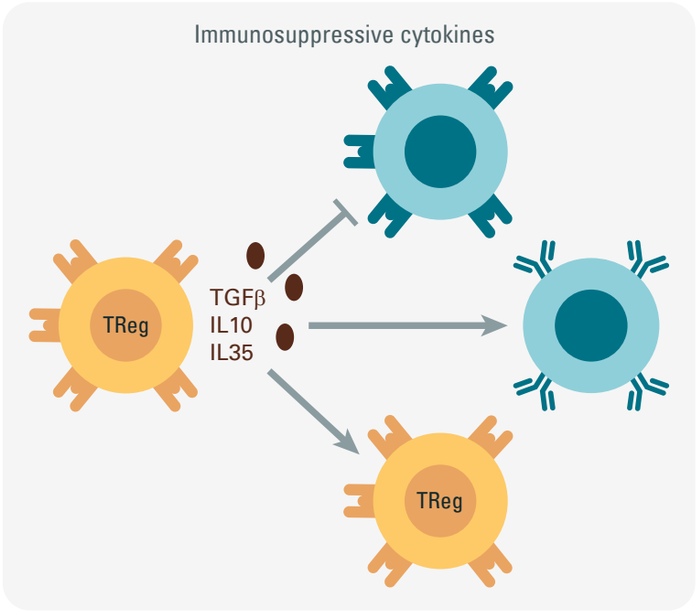
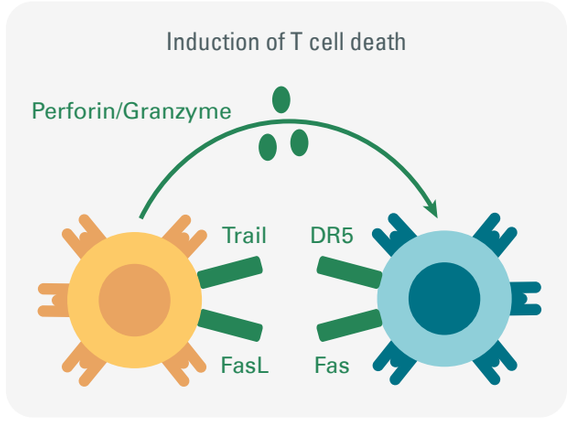
REGULATORY T-CELLS IN SELF TOLERANCE









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CELLULAR AND MOLECULAR MECHANISMS OF AI DISEASES

Innate immunity components

If a dysregulation of the adaptive immune response is involved in the development of autoimmune diseases, innate immune components which precede and prime the adaptive response also play a role in their pathophysiology.

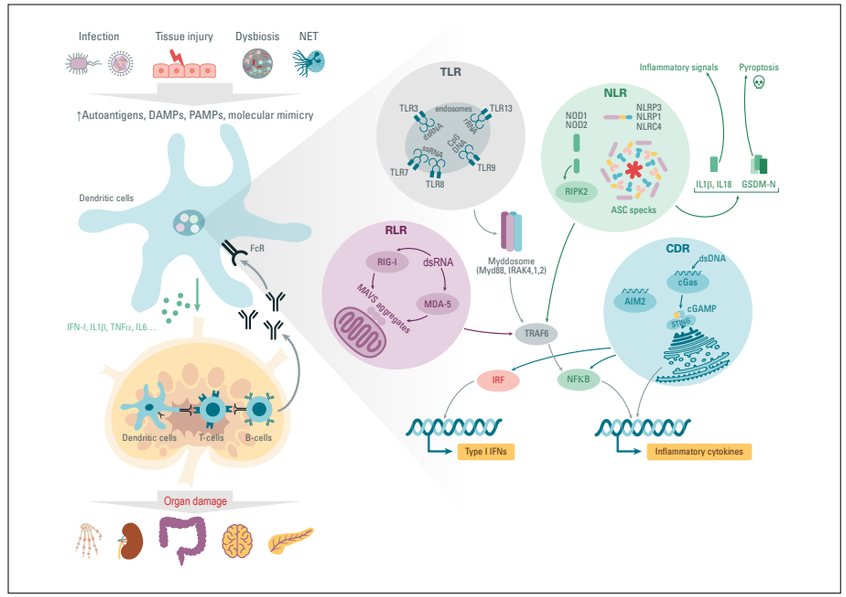
Innate immune cells such as dendritic cells express a broad spectrum of sensors, called Pattern Recognition Receptors (PPR), that are either located at the plasma membrane or in the cytoplasm (77). PPRs recognize pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). Membrane receptors include Toll-like receptor 4, 1, or 2 (TLR), C-type lectin receptors (CLRs) and sialic acid binding Ig-like lectins (Siglecs). Endosomal sensors from the Toll-like receptor (TLR) family include TLR3 for double-strand RNA, TLR7 and TLR8 for single-strand RNA, and TLR9 for DNA (78) (79). Cytosolic sensors include Rig-1 like receptors (RLR) such as the helicases RIG-I for uncapped 5'-triphosphate RNA and MDA5 for long dsRNA (80); the cytosolic DNA Receptors (CDR) such as the double strand DNA cGAS enzyme or AIM2 (81), as well as cytosolic NOD-like Receptors (NLR) including NODs, NLRPs or NLRCs (82) (83). When activated, they all contribute to the transcriptional gene expression encoding proinflammatory cytokines such as TNFa, IL6, or IL1b, and type 1 IFN. These cytokines play an essential role in bridging innate and adaptive immune responses. Activation of the TLRs, RLRs, or CDRs triggers signaling cascades which involve a variety of intracellular mediators such as Myd88, TRAF6, IRAKs, NFKB, or IRFs, and convey cytokine transcriptional programs.

Inflammasome involves various NLR and contributes to the generation of the adaptive immune response through proteolytic maturation of inflammasome dependent cytokines (IL-1β and IL-18). This enzymatic cleavage relies on ASC-speck formation and the subsequent Caspase-1 activation, and ultimately leads to an inflammatory programmed cell death called pyroptosis (84).

Numerous studies in animal and human models have revealed the crucial role of innate immune sensors in autoimmune pathogenesis. For example, lupus-mice models showed disease regression when IFNAR or TLR7 and 9 genes were deleted (85) (86). In human, the activation of MDA5 and RIG-1 has been reported in some SLE patients (87). Moreover, dysfunctions of DNAse such as TREX1 contribute to various autoimmune diseases by the accumulation of DNA and subsequent PRR activation (88). In addition, Neutrophil Extracellular Traps have been shown to induce TLR activation and type I IFN response (89). Inflammasome dysregulations have been associated with SLE and RA diseases (90). Furthermore, interaction between NETs and inflammasomes has been documented in SLE patients. SLE associated autoantibodies such as anti-dsDNA were shown to activate inflammasome in such patients (90).

Thus, innate immune components are now considered as major players in the innate immunity contribution to autoimmune pathogenesis.

For more information regarding general immunology and immune signaling pathways, please read our Immunology guide.



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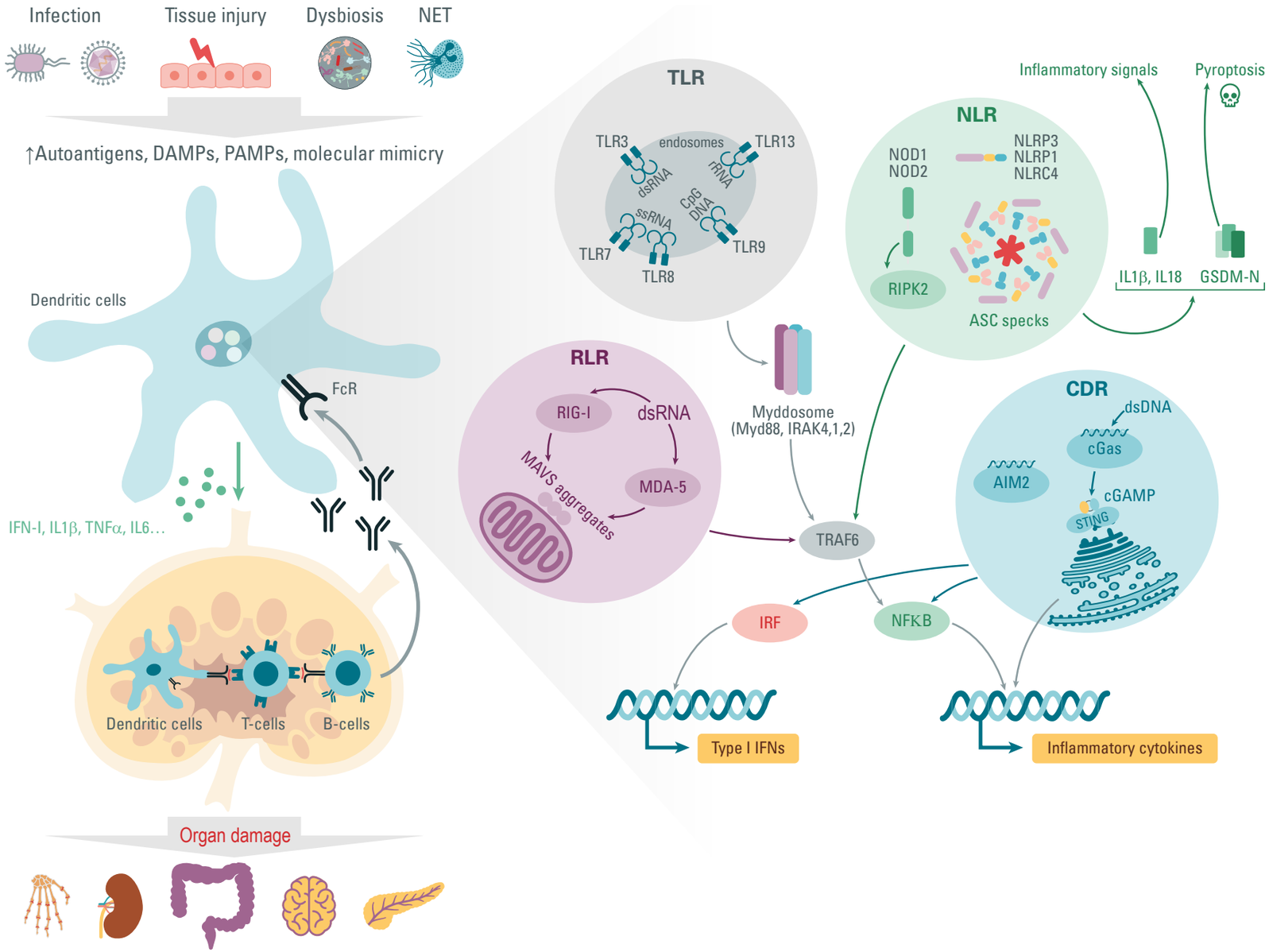

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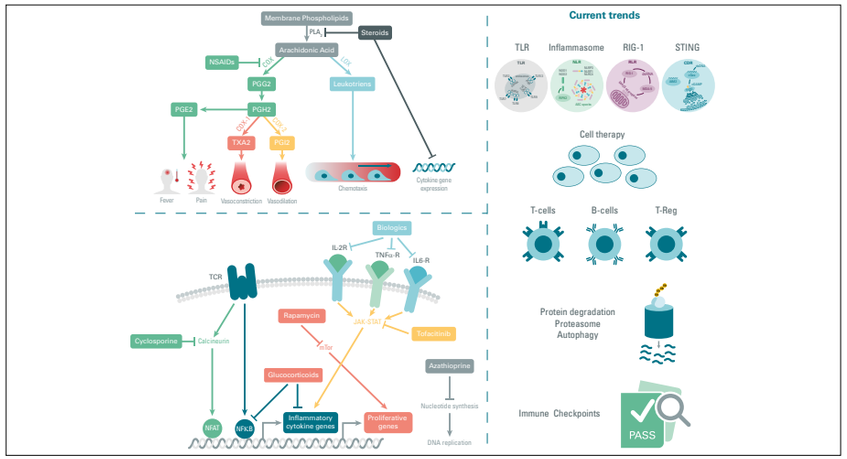
THERAPEUTIC STRATEGIES IN AUTOIMMUNE DISEASE TREATMENT

Nonsteroidal anti-Inflammatory drugs(NSAIDs)

This class of molecules (e.g. ibuprofen) reduces inflammation by blocking the enzyme cyclooxygenases (COX-1 and COX-2) or COX-2 selectively (e.g. Celecoxib), and the production of thromboxane A2 and prostaglandins.



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THERAPEUTIC STRATEGIES IN AUTOIMMUNE DISEASE TREATMENT

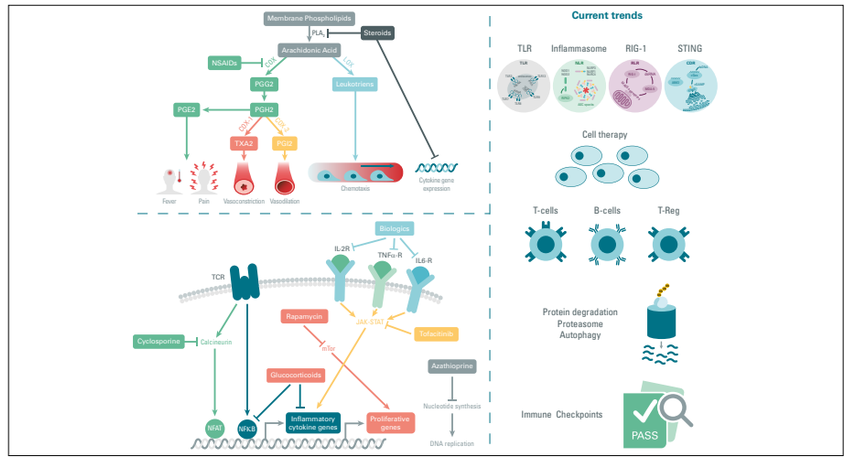
Immunosuppressive drugs

SMALL MOLECULES

- Glucocorticosteroids (e.g. cortisone) reduces inflammation by blocking Phospholipase A2 and the production of prostaglandins, leukotrienes and cytokines.
- Janus kinase inhibitors (e.g. tofacitinib) inhibit signal transduction from cytokine receptors.
- Calcineurin inhibitors (cyclosporine) inhibit the cytoplasmic phosphatase calcineurin, leading to reduced production of IL-2, IL-3, and interferon-γ.
- mTOR inhibitors (e.g. Rapamycin) inhibit intracellular FKBP-12 protein and reduce cytokine-induced T-cell proliferation.
- IMDH inhibitors (e.g. azathioprine) block nucleotide synthesis and lymphocyte proliferation.
- BTK inhibitors (e.g. Ibrutinib) inhibit BCR signaling and B-cell proliferation.

BIOLOGICS

- Anti-IL2 receptor antibodies (e.g. basiliximab, daclizumab) inhibit T cell proliferation by blocking the binding of IL-2.
- Anti-soluble TNFa antibodies (e.g. infliximab, Adalimumab) or fusion TNF receptor recombinant protein (etanercept) block TNFa pro inflammatory effects.
- Anti-IL6 receptor antibodies (e.g. tocilizumab) inhibit IL6 pro inflammatory effects.
- Anti-CD20 antibodies (e.g. Rituximab) induce B-cell apoptosis.
- Anti-CD49 (cell adhesion molecule α4-integrin) antibodies (Natalizumab) block immune cell adhesion and their ability to pass through the intestinal barrier or blood brain barrier.
- Fusion CTLA-4 recombinant protein (Belatacept) inhibits T cell activation by blocking the B7 costimulatory molecule binding to CD28.
- Anti-VLA-4 antibodies (e.g. Natalizumab) block the VLA-4/VCAM-1 interaction and prevent immune cell infiltration.



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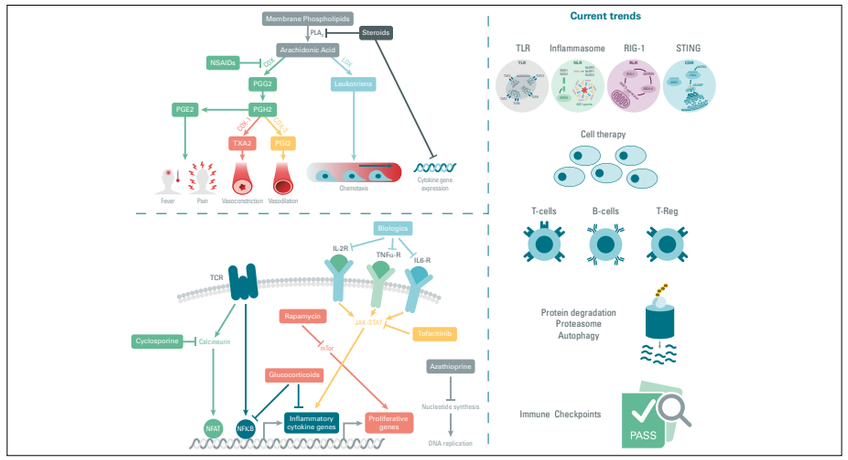
THERAPEUTIC STRATEGIES IN AUTOIMMUNE DISEASE TREATMENT

Promising new therapeutic areas

Several new cellular and molecular targets have recently emerged as potential new areas for therapeutic intervention to treat auto-immune diseases. The targeting of innate immune components like inflammasome, TLR, or RLR is currently under scrutiny. Investigations aiming to restore immunosuppressive functions of T-Reg subsets or dampen B and T-cell activity may open up another area for future therapy, for example by targeting Immune checkpoint molecules. Intracellular pathways related to protein degradation (e.g. proteasome, autophagy) are being explored as well. Progress in cell therapies is also considered as a viable therapeutic axis in autoimmunity. Overall, improvements in autoimmune disease management may well result from the fast-growing understanding being achieved in the immunology field.



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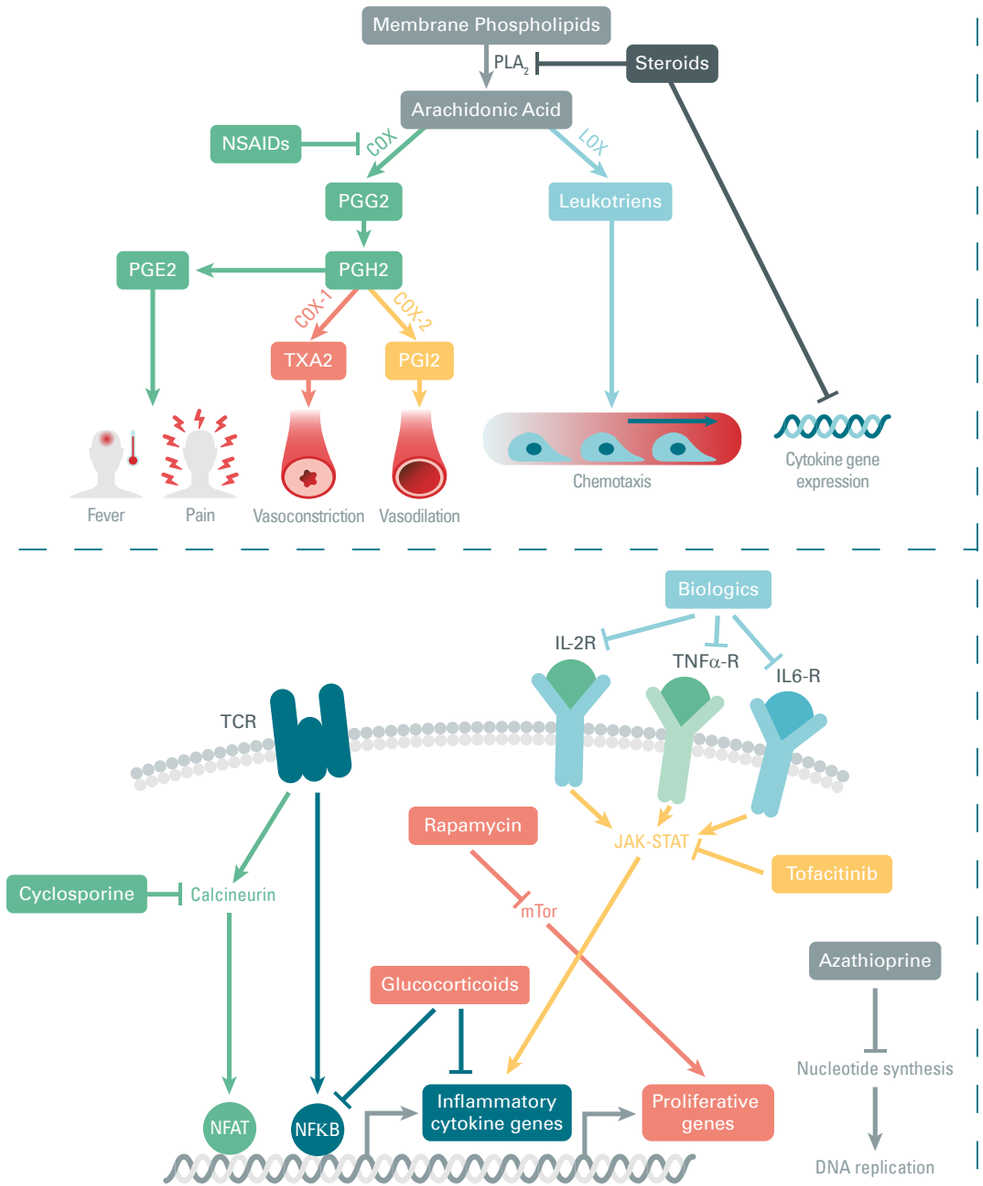

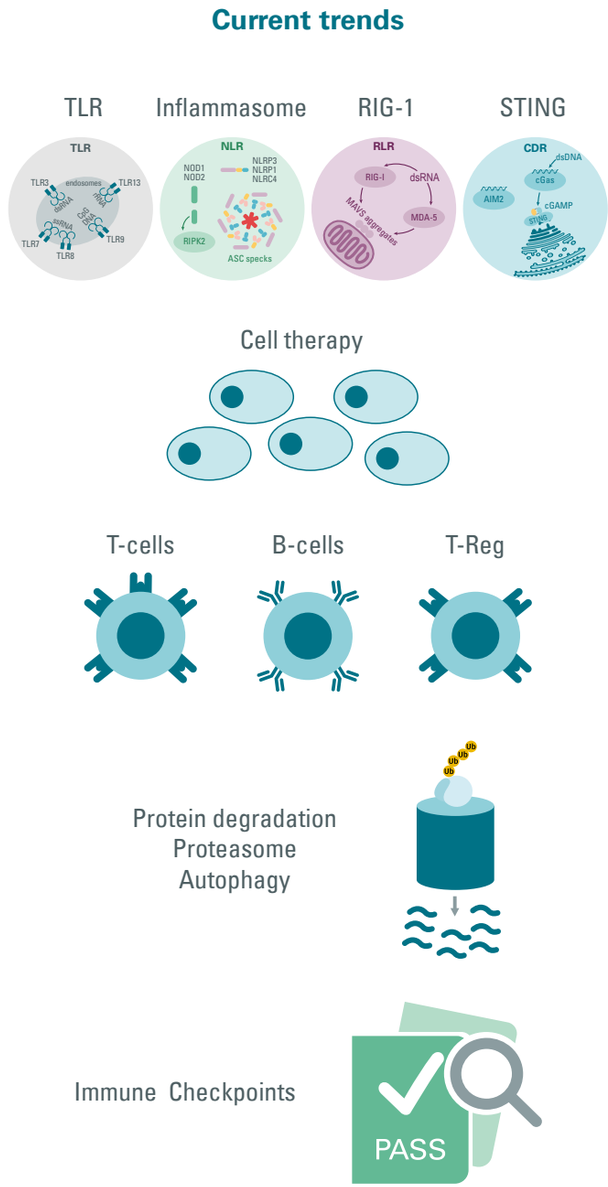

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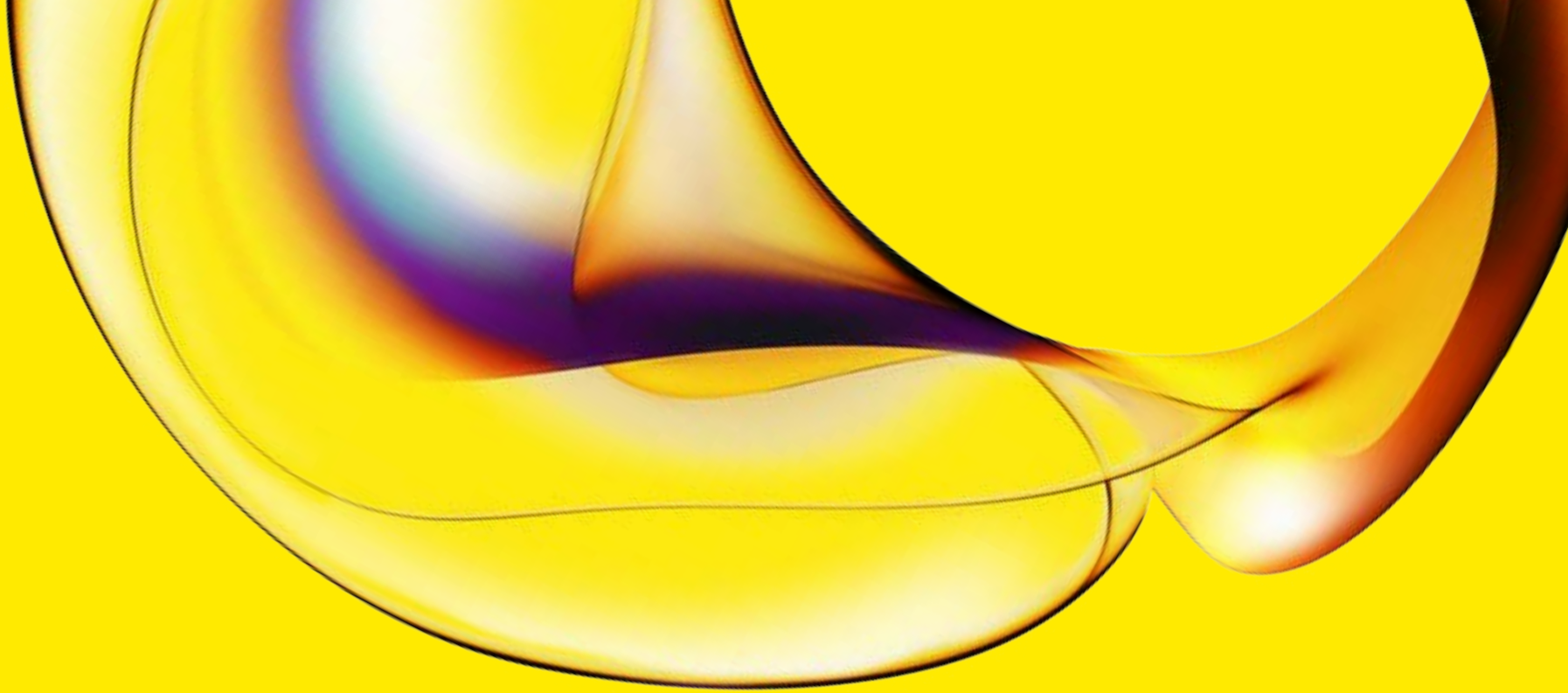

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