Centrum pro výzkum toxických látek v prostředí

Speciální Imunotoxikologie
Vliv chemických látek na Imunitní systém - 1

Luděk Bláha

blaha@recetox.muni.cz

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Jakými mechanismy ovlivňují chemické látky I.S. ?

- I.S. - složitá struktura, fungování regulace
  - Působení ch.l. na I.S. - neexistuje jeden jednoduchý mechanismus
  - Zpravidla se uplatňuje jeden či více různých procesů

1) Význam prostorových změn molekul (proteinů)
  - změny povrchu proteinů
  - ovlivnění funkcí APC a lymfocytů
  - vyvolání autoimunitních reakcí
  - imunomodulace: reaktivní chemické látky v těle

2) (modulační) efekty na molekulární a enzymové úrovni
  - narušení výkonných funkcí (enzymy, oxidázy …)
  - narušení syntézy DNA, proteinů
  - ovlivnění signálních drah a molekul (receptory, cAMP, Ca2+)
  - modifikace membrány (signalizace …)

3) Nepřímá modulace systémů, které „řídí" a souvisí s IS
  - Neurohumorální regulace
  - Obecný stres apod.

Ad 1) Význam prostorových změn molekul v I.S.

- - Chemická látka **pozmění mk** v těle
  - rozpoznání B-b. / aktivace T-b.
    → **auto Ab proti vlastním Ag**
- - Chemická látka **pozmění MHC na APC**
  → Ab bez indukce Ag (i proti vlastním Ag - také auto Ab)
- - Chemická látka pozmění TCR
  - → T-b. nerozezná cizorodou strukturu NEBO T-b. chybně rozezná vlastní strukturu
-

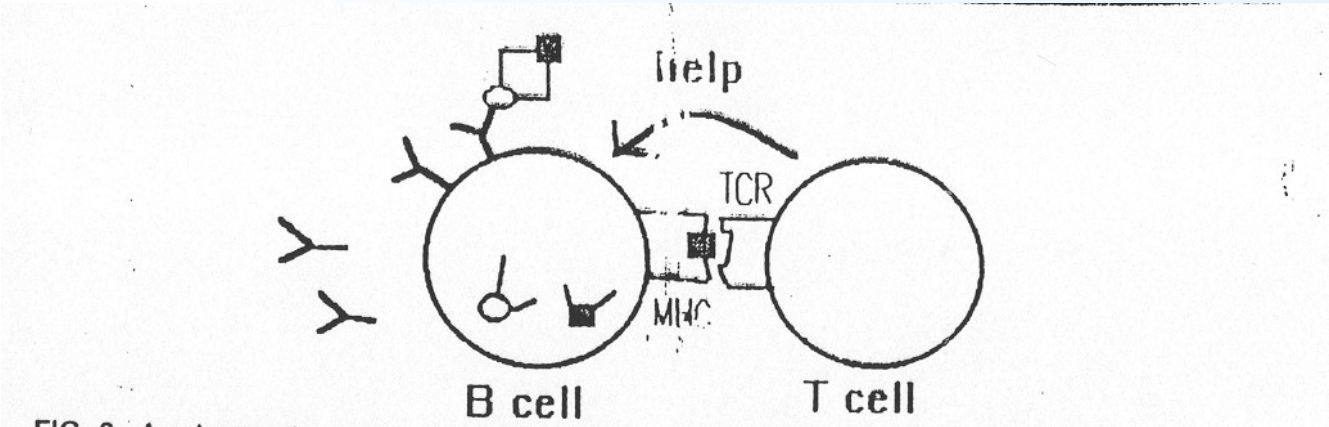
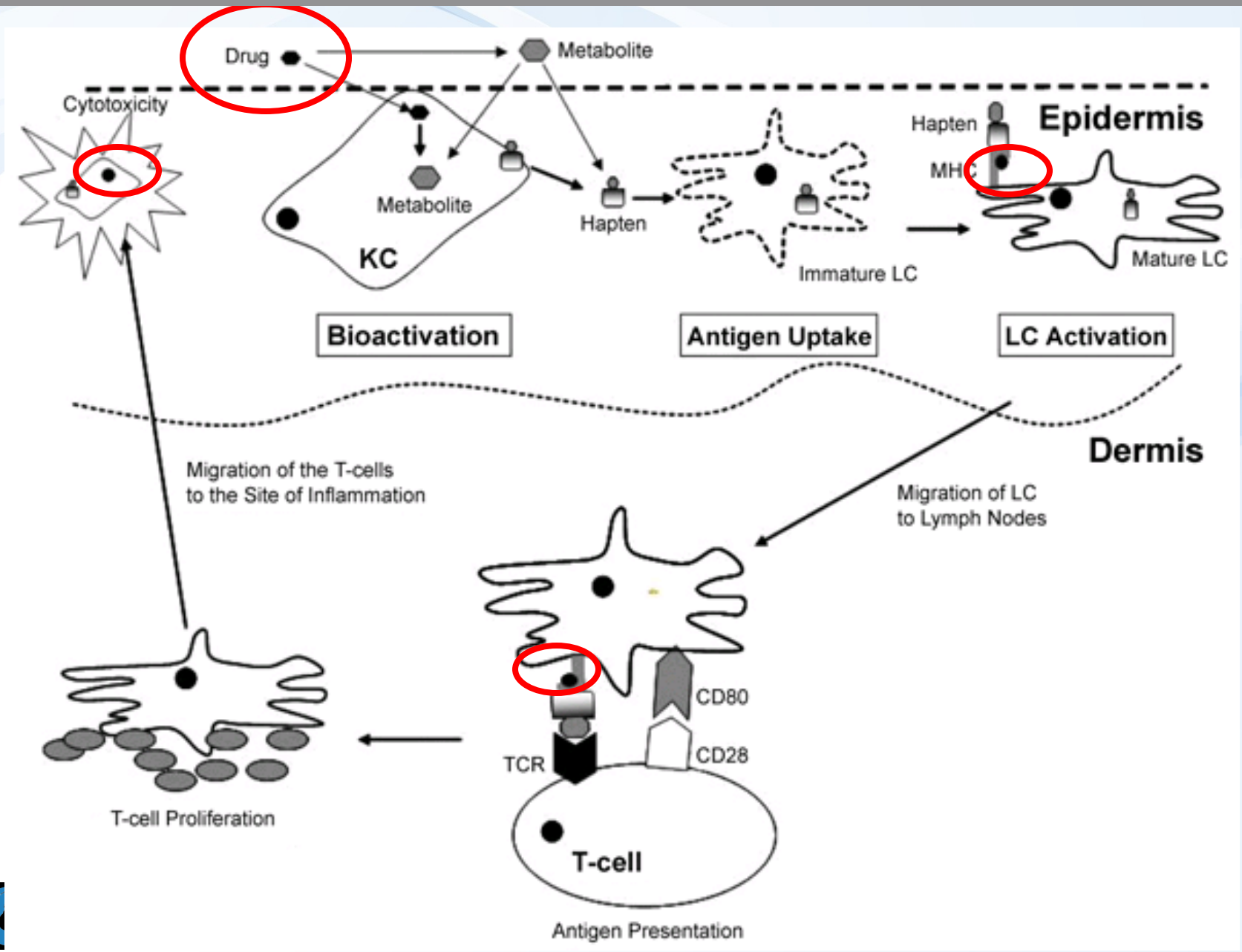


FIG. 2. A scheme showing how a drug might induce a response to unmodified self. The *small open circles* represent self while the *small filled squares* represent the drug-induced determinant to be recognized by the T cell. Otherwise the scheme is identical to that shown in Fig. 1. Based on ideas from refs. 41 and 42.

Význam prostorových molekul v I.S.

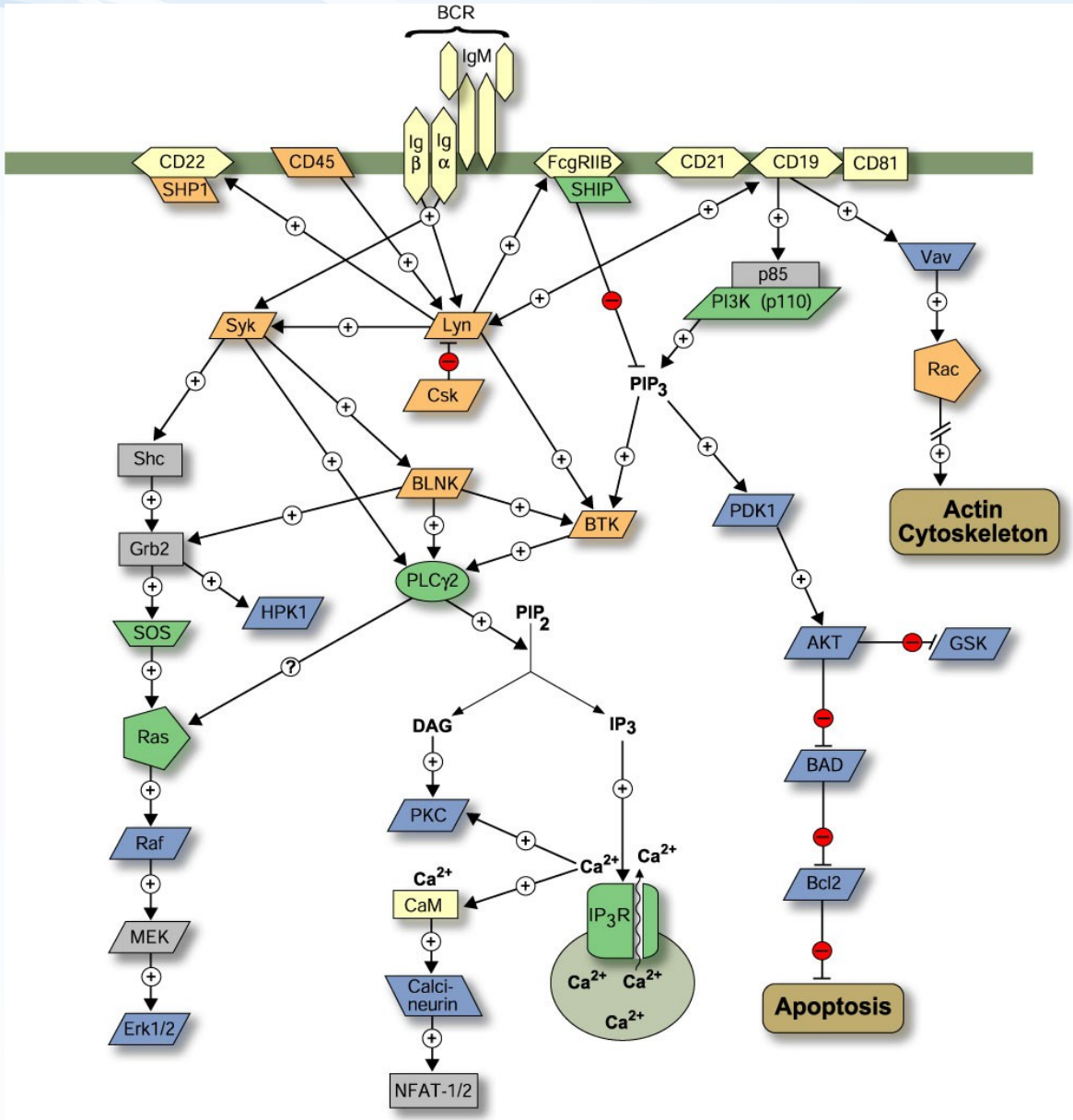


Narušení regulací v I.S.

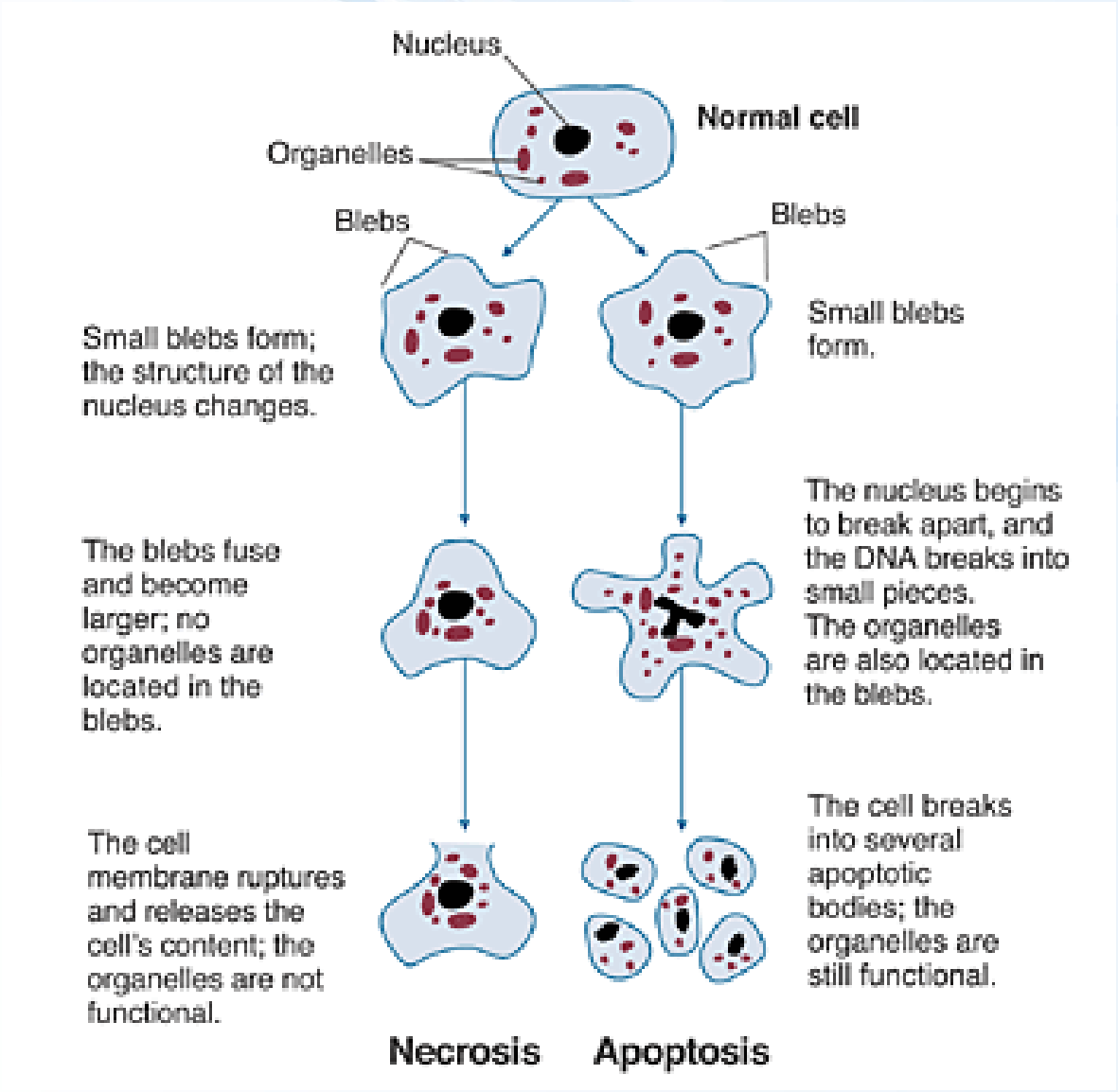
– neurohumorální řízení
- význam apoptozy

Ad 2) Narušení regulací v buňkách I.S.

- Regulace obecně založeny na velmi malých změnách (např. nízké koncentrace hormonů, Ca^{2+} …)
- Zásah do regulačních procesů → velké dopady in vivo
- Složité procesy (zatím málo prostudované): *Regulace a toxicita: v současnosti velká pozornost výzkumu*



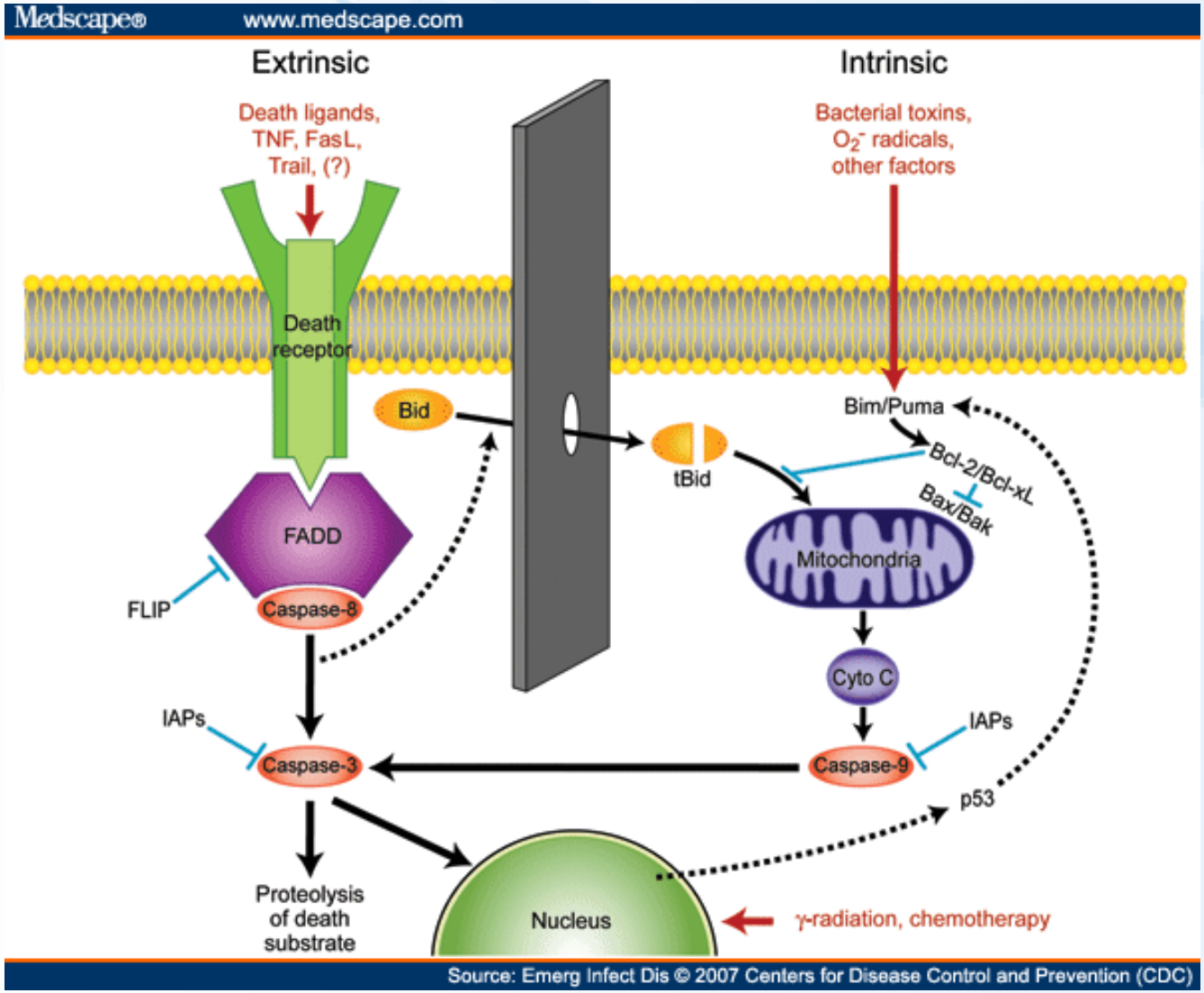
Apoptoza vs. Nekroza



Apoptoza – centrální proces v regulaci I.S.

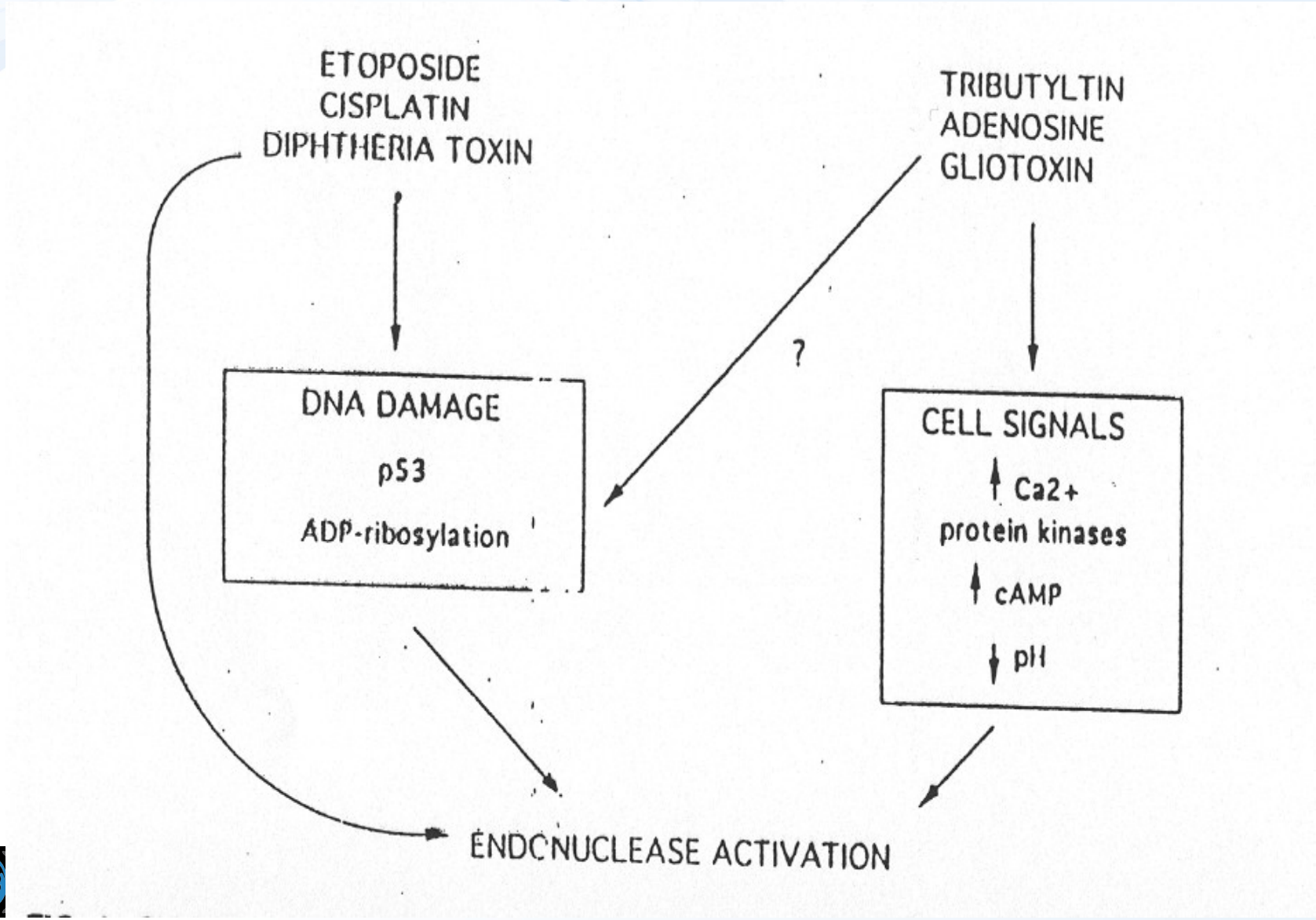
Velký význam apoptozy v I.S. (příklady):

- Zrání T-bb.
- Klonální proliferace
- Působení Tc, NK bb.



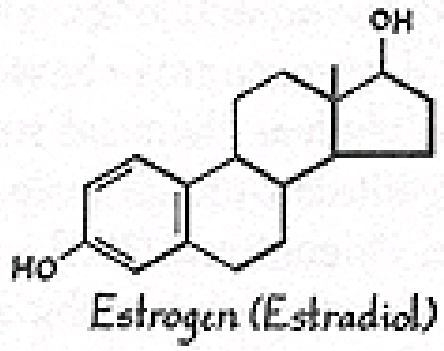
Centrum pro výzkum toxických látek v prostředí

Působení známých „imunotoxinů“ → modulace apoptozy

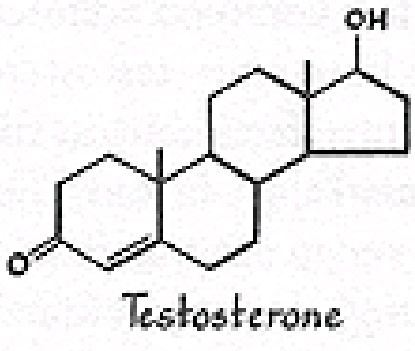


Působení látek na neuroendokrinní systém

- Imunitní systém je pod kontrolou NEUROENDOKRINNÍHO SYSTÉMU
  - Z hlediska toxicity - význam zejména (nízkomolekulární) hormony
  - interference toxikantů (strukturně „blízké" ch.l.)
    - Steroidy: estrogeny, androgeny, kortikoidy
    - Thyroidní hormony a další

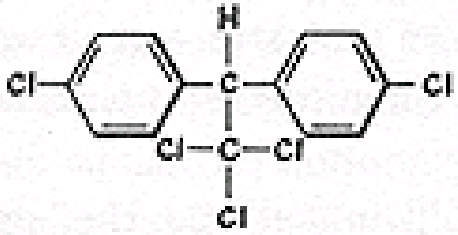


Estrogen (Estradiol)

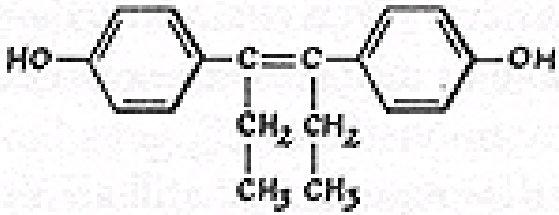


Testosterone

thyroxin

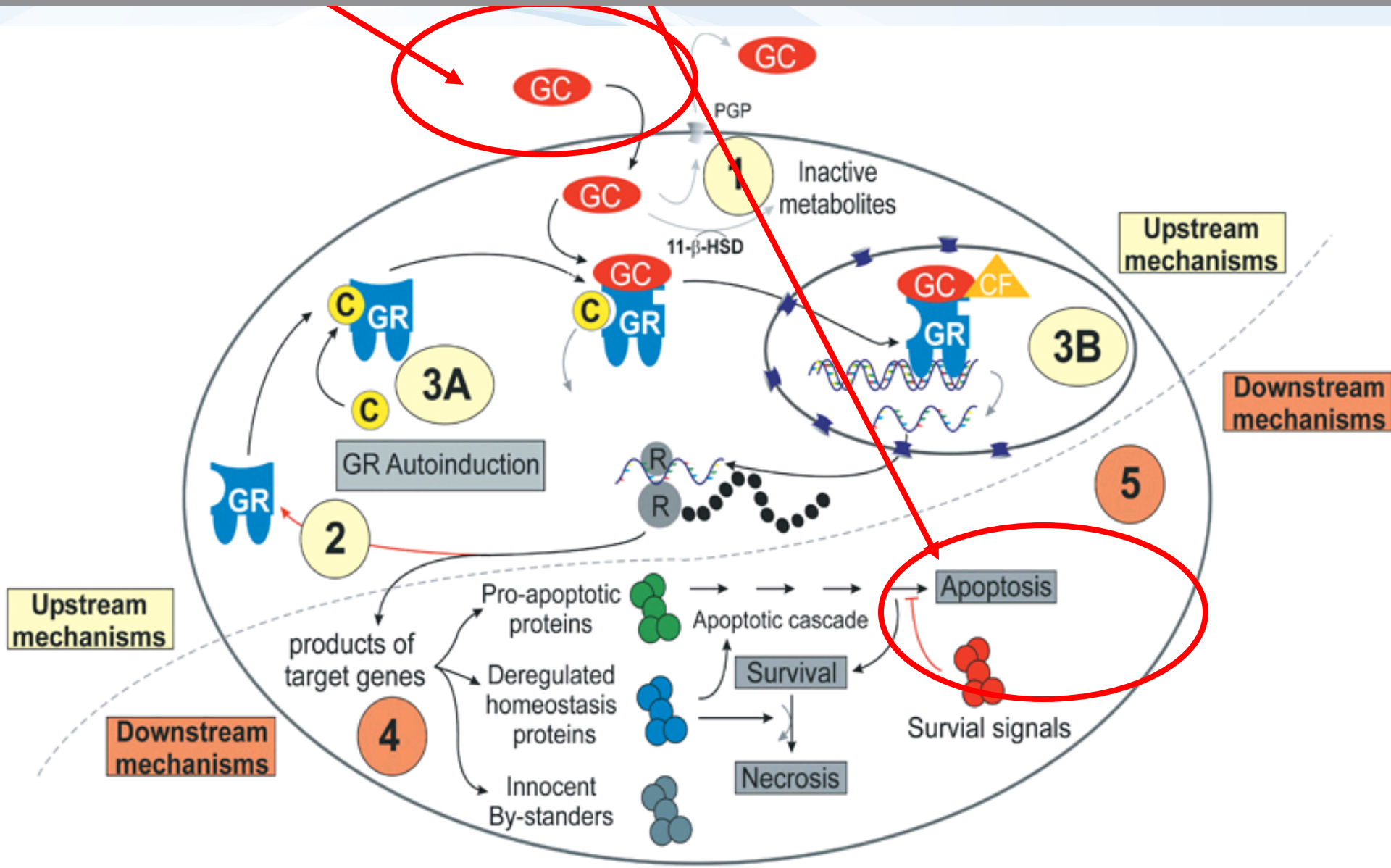


DDT



Diethylstilbestrol

Glukokortikoidy a apoptoza

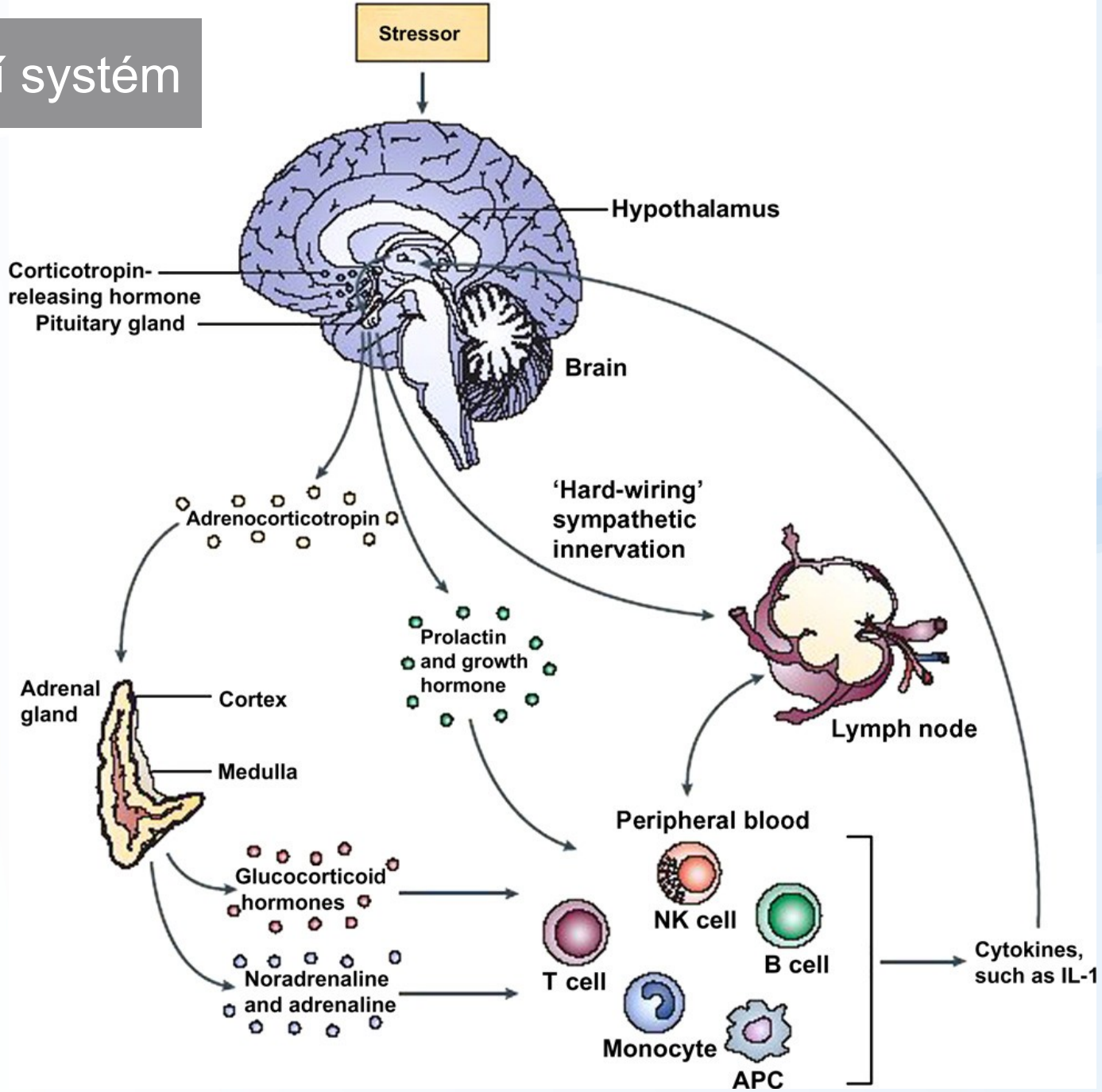


Stres a význam pro I.S.

Stres a Imunitní systém

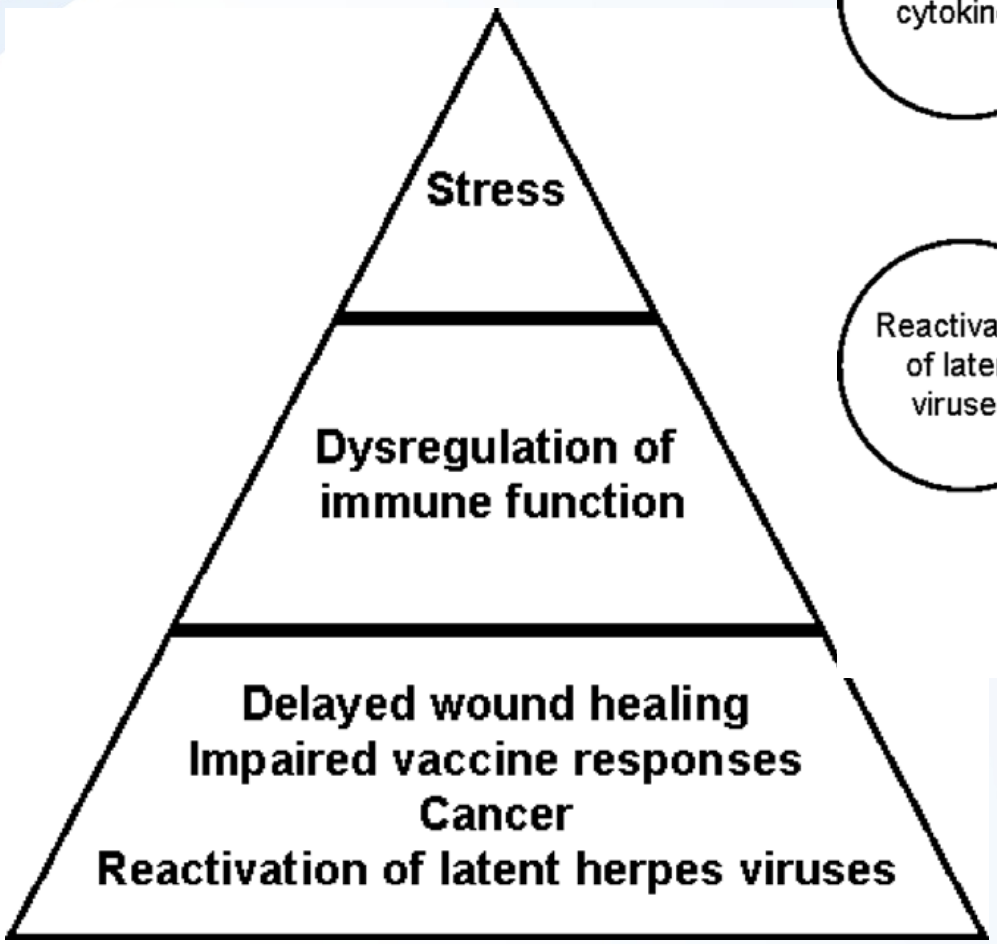
Obecný i chemický stres
→ Ovlivnění CNS a Humorální regulace

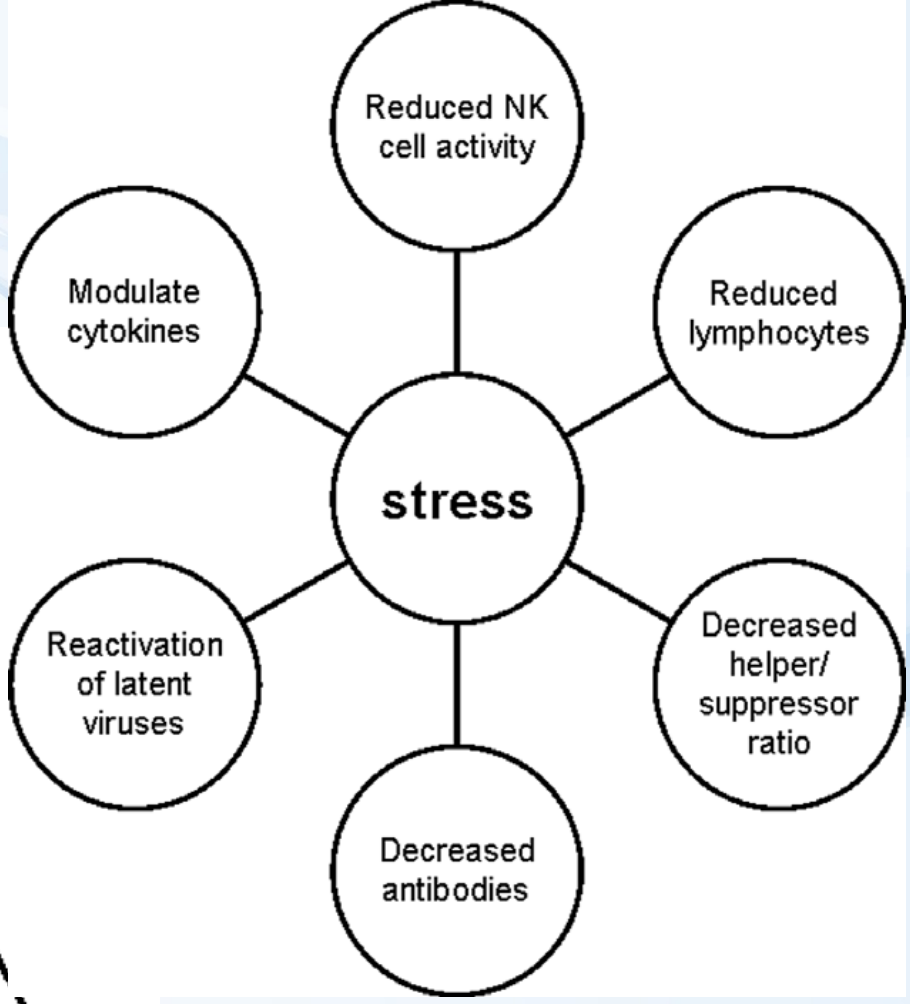
→Stresové hormony:
- Adrenalin
- Steroidy – KORTIZOL !



Centrum pro výzkum toxických látek v prostředí

Význam stresu v supresi I.S.





Reaktivní toxikanty

→ Chemikálie a hypersensitivity

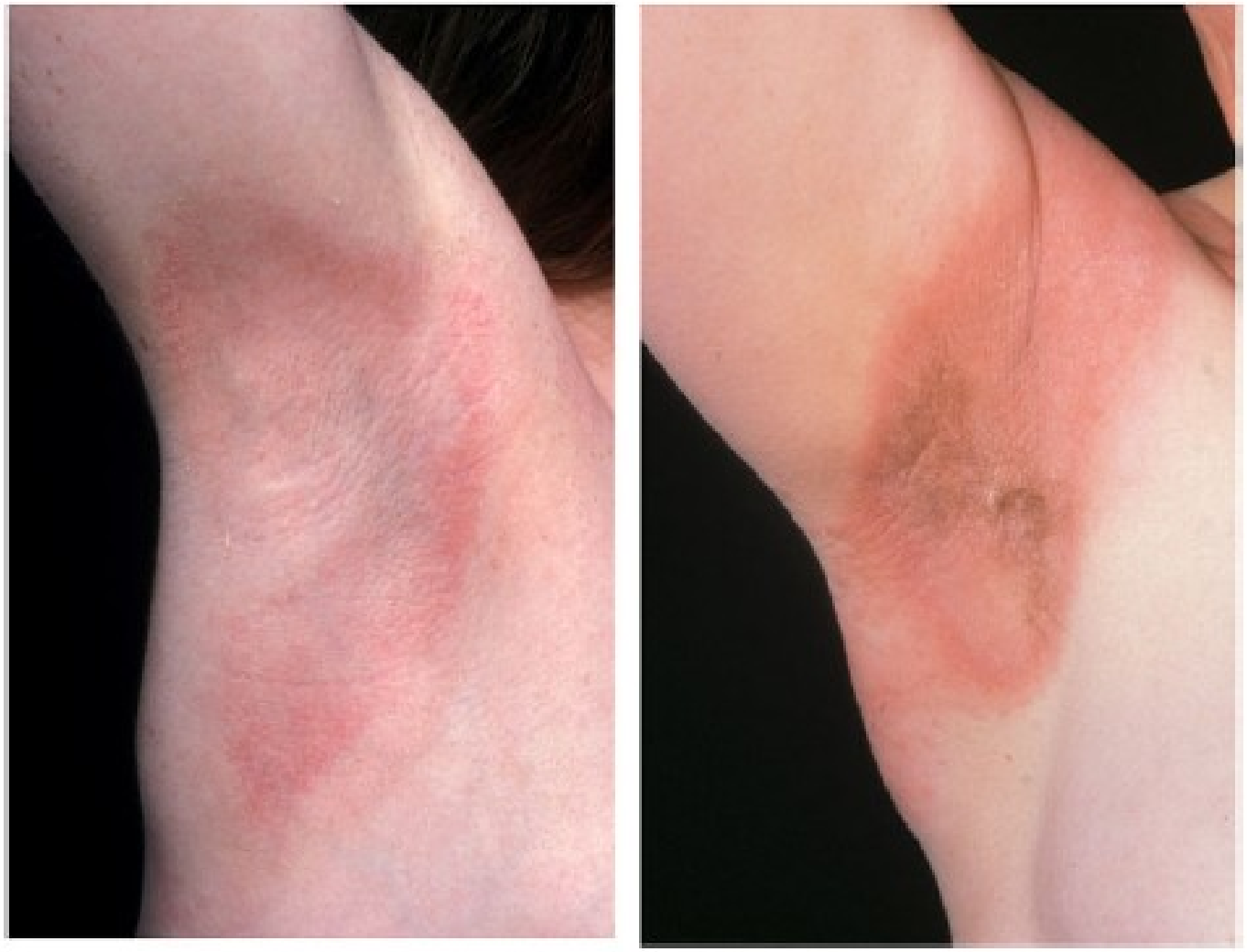
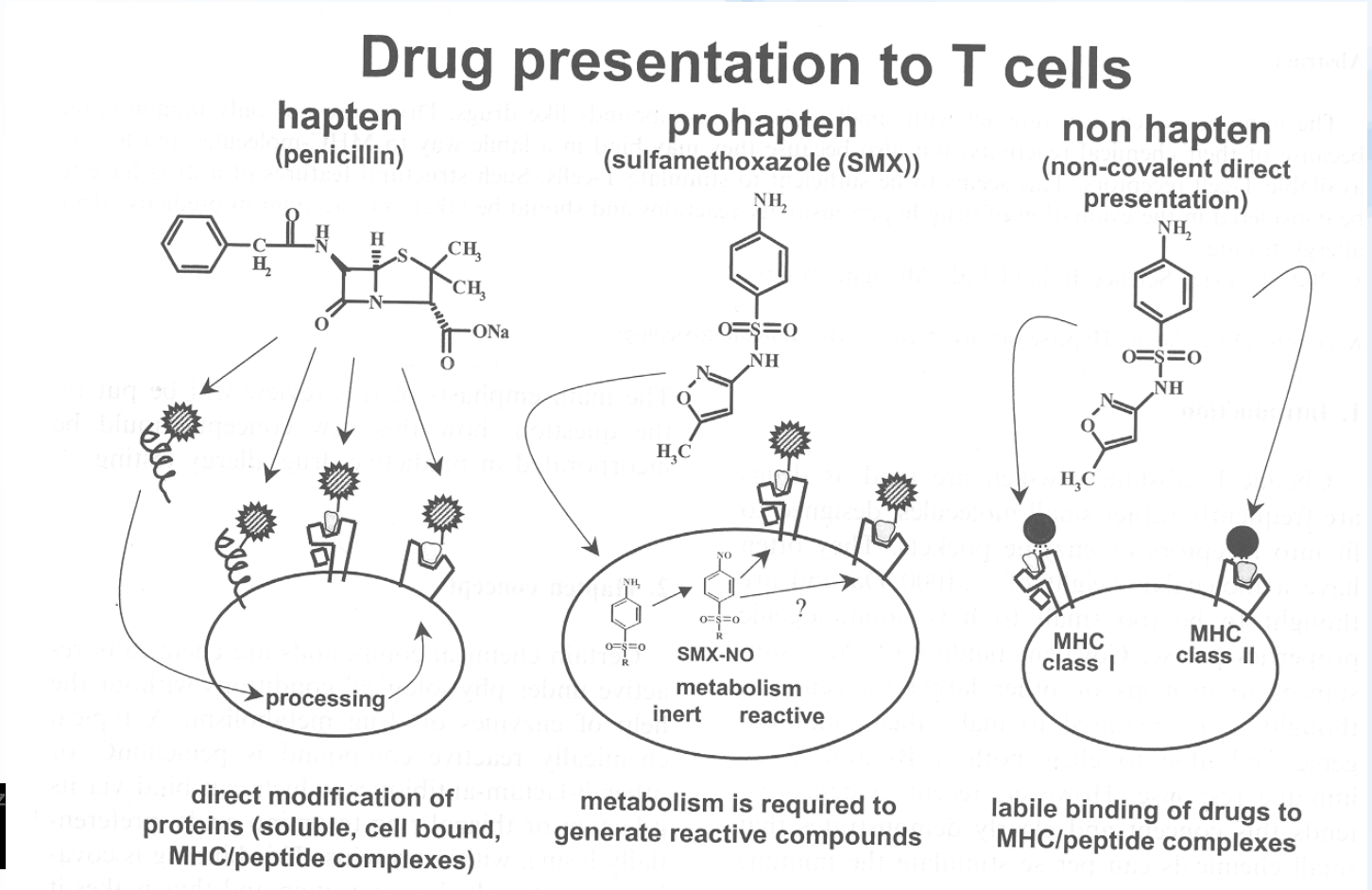


FIG. 1. ACD of axillae associated with the use of personal care products.

Reaktivní látky a vznik antigenů

- **Nízkomolekulární reaktivní látky (LMW)**
  - přímá reaktivita NEBO po aktivaci
  - konjugace s endog. proteinem -> imunogen
  - nebo i nekovalentní vazba s MHC



Centrum pro výz
toxických látek
v prostředí

Fáze senzitizace na kůži

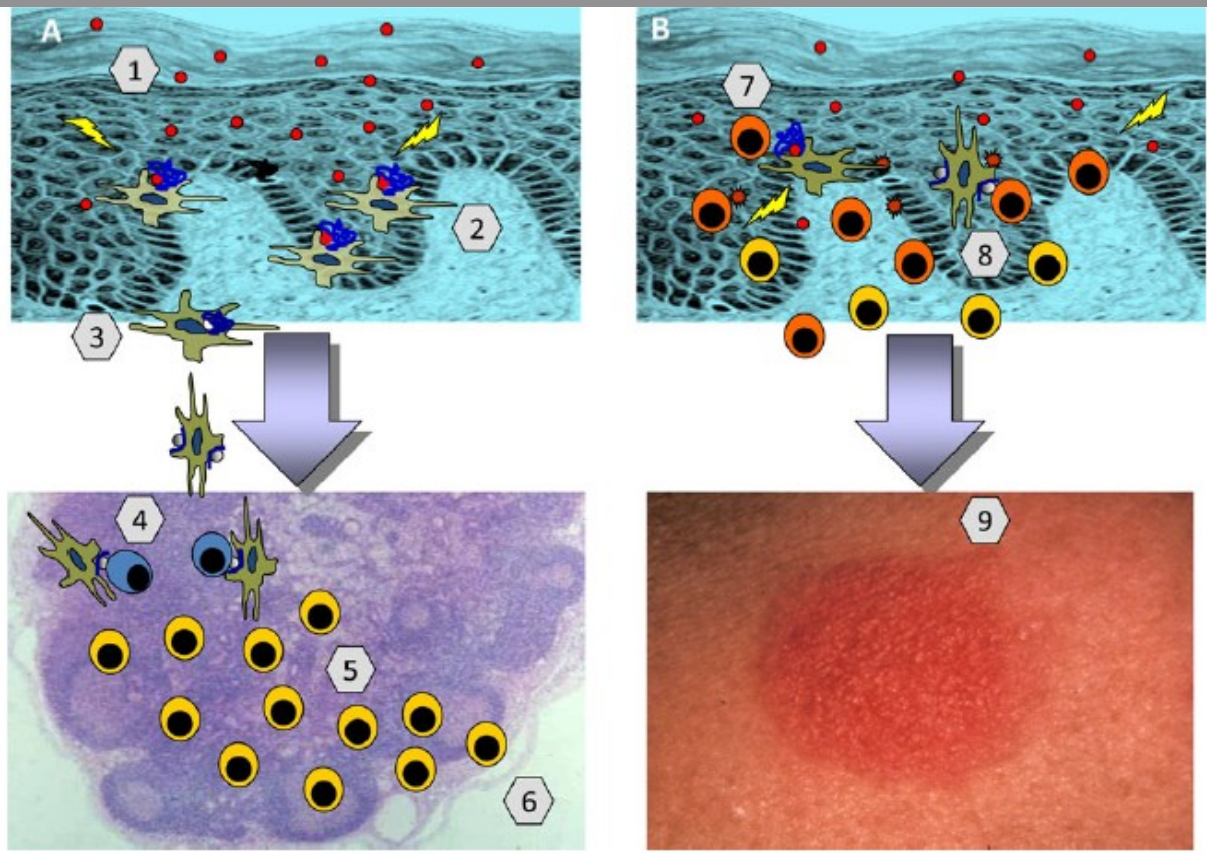


FIG. 2. The induction phase of skin sensitization (A). Chemical allergens gain access to the viable epidermis and associate in stable fashion with protein (1). There is the local release of various proinflammatory cytokines and other ''danger signals'' that are required to support immune activation and the engagement of DC (2). LC (and other cutaneous DC) are activated and recognize, internalize, and process haptenated protein. These cells transport antigen from the skin to draining lymph nodes, via the afferent lymphatics, during which time they become activated and differentiate into mature, antigen-presenting cells (3). Haptenated peptides are presented to naive, antigen-responsive T lymphocytes (4). The antigen-driven activation of responsive cells is associated with rapid turnover and selective clonal expansion of antigen-specific T lymphocytes (5). The expanded population of primed antigen-specific T lymphocytes (effector and memory T lymphocytes) disseminates into the peripheral circulation (6). At this point, sensitization has been acquired. The elicitation of ACD (B). Elicitation is triggered by exposure of the now-sensitized subject, at the same or a different skin site, to the same chemical allergen (7). Allergen-specific T lymphocytes accumulate at the site of encounter with the chemical (8). T lymphocytes become activated and are stimulated to release cytokines, chemokines, and other inflammatory mediators that act in concert to draw in other leukocytes and drive the cutaneous inflammatory reaction that is characterized by erythema, edema, and visiculation and that is recognized clinically as ACD (9).

Příklady reaktivních látek & imunotoxinů

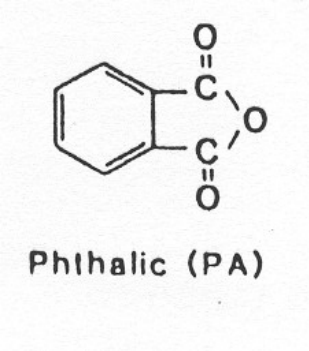
- **Pryskyřice, součásti lepidel - ALERGIE**

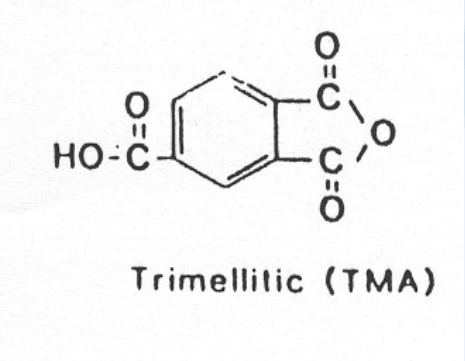
- Typ I- rychlá reakce závislá na IgG
  - anhydridy kyselin, polyisokyanaty

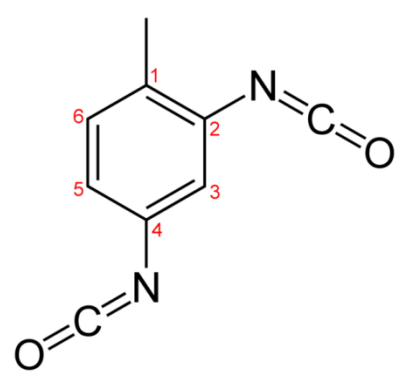
- Typ II- vyžad. IgM a IgG + aktivace C
  - anhydrid kyseliny trimelitové (TMA)

- *(Typ III- imunonkomplexy - aktivace C)*

- Typ IV- zpožděná hypersensitivita (DTH)
  - toluen-diisokyanat







Centrum pro výzkum toxických látek v prostředí

Léčiva a hypersensitivita

→ AUTOIMUNITY: např. LUPUS - velké množství léčiv
→ ALERGIE: bleomycin, metotrexat, peniciliny, ampiciliny …

Table 1
Drugs definitively associated with drug-related lupus[+] (a) and other drugs associated with drug-related lupus and currently in use* (b)

(a) *Drugs with drug-related lupus*

Chlorpromazine
Hydralazine
Isoniazid
Methyldopa
Minocycline
Procainamide
Quinidine

(b) *Drugs with drug-related lupus and currently in use*

Acebutolol
Acecainide
Allopurinol
Aminoglutethimide
Amoproxan
Anthiomaline
Anti-tumor necrosis factor alpha
Atenolol
Benoxaprofen
Captopril
Carbamazepine
Chlorprothixene
Chlorthalidone
Cimetidine
Cinnarazine
Clonidine
Danazol
Diclofenac
1,2-Dimethyl-3 hydroxy-pyride-4-1
Diphenylhydantoin
Disopyriamide
Enalapril
Estrogens
Ethosuximide
Ethylphenacemide
Gold salts
Griseofulvin
Guanoxan
Ibuprofen
Interferon alpha
Interferon gamma
Interleukin 2
Labetalol
Leuprolide acetate
Levadopa
Levomeprazone
Lithium carbonate
Lovastatin
Mephenytoin
Methimazole
Methlysergide*
Methylthiouracil*
Metoprolol
Metrizamide
Minoxidil
Nalidixic acid
Nitrofurantoin
Nomifensine*
Oxyphenisatin
Oxyprenolol
Para-amino salicyclic acid
Penicillamine
Penicillin
Perazine
Perphenazine
Phenelzine
Phenopryazone*
Phenylbutazone*
Phenylethyacetylurea*
Phenytoin
Practalol*
Prazosin
Primidone
Prindolol
Promethazine
Propafenone
Prophythiouracil
Propranolol
Psoralen*
Pyrathiazine
Pyrithoxine
Quinine
Reserpine
Spironolactone
Streptomycin
Sulindac
Sulfadimethoxine
Sulfamethoxypyridazine
Sulfasalazine
Tetracyclines
Tetrazine
Thionamide*
Thioridazide
Timolol eyedrops
Tolazamide
Tolmetin
Trimethadione

[+] Substantial observations and studies.
* A few are single case reports but the majority represent good clinical observations.

Autoimunity a průmyslové chemické látky

formaldehyd, chloramin, diazoniové soli, rozpouštědla thiomočoviny …

TABLE 1. *Possible relationships between human sclerotic and lupus-like diseases and environmental chemical exposures*

Chemical	Reference	Observation
Occupational		
VC	Lange et al. (8)	Skin sclerosis
	Ward et al. (9)	Lung fibrosis
	Black et al. (10)	Nervus system paresthesia
		Vessels—capillary
		Inflammation, intimal fibrosis
		Thrombocytopenia
		Symptoms—fatigue, cold burning pain, emotional instability, loss of libido, impotence
		Autoantibodies—not detected
Tetrachloroethylene	Sparrow (70)	19-Year-old male dry cleaner, 4 years, elevated ANA titers, systemic sclerosis
CE	Reinl (11)	24-Year-old woman, degreasing—scleroderma
Solvents	Yamakage and Ishikawa (14)	7/9 Patients in Japan—Raynaud's phenomenon, sclerosis; 6 had lung fibrosis
Solvents, toluene, xylene, white spirit	Walder (12, 13)	6/7 Solvent workers in Australia; added 5 in 1983—scleroderma
Carbon tetrachloride and TCE	Saihan et al. (71)	43-Year-old male, neuropathy, Raynaud's phenomenon, sclerosis
Organic solvents	Sverdrup (72)	Scleroderma in 8/9 manufacturing workers
TCE	Lockey et al. (15)	47-Year-old female, fatal scleroderma 6 months after 2.5-hr dermal exposure to TCE

Autoimunity a chemické látky

Table 1. Substances Associated with Autoimmunity in Humans and the Animal Models Used To Examine Disease Mechanisms

drugs/chemicals[a]	human disease	animal model[b]	refs
drugs (procainamide)	drug-induced lupus	mouse	
		• central tolerance	*16−19*
		• DNA methylation	*10−12*
silica/asbestos	lupus, systemic sclerosis, rheumatoid arthritis, vasculitis	mouse	
		• C57Bl/6	*38*
		• lupus-prone	*32−34*
adulterated rapeseed oil	Toxic Oil Syndrome	mouse	
		• B10.S	*42, 43*
		• lupus-prone	*44*
iodine	thyroiditis	mouse	
		• NOD-H-2h4	*119*
trichloroethylene	hypersensitivity skin disorder, scleroderma, hepatitis	mouse	
		• lupus prone	*153−155*
		• lupus prone (prenatal)	*156*
metals (Hg, Au, and Ag)	nephropathy, autoantibodies	mouse	
		• B10.S	(Hg) *46, 47* (Ag) *47, 49* (Au) *48*
		• lupus-prone	*76, 108*
TCDD, dioxin[c]	antinuclear autoantibodies	mouse	
		• GVHD[d]	*149*
		• EAE[e]	*150*
		• neonatal exposure	*152*
pesticides/fungicides (hexachlorobenzene)	chronic inflammatory response	rat	*162*
		mouse	
		• lupus prone	*164*
mineral oil (pristane, TMPD[f])	chronic inflammatory response (follicular lipidosis)	mouse	
		• C57BL/6, BALB/c	*134, 139*
		• lupus prone	*135*

[a] Where multiple examples of a drug or chemical exist, only those discussed in the text or cited in the accompanying publications are noted. [b] In many studies examining the lupus-inducing potential of toxins, mouse strains that are prone to develop lupus spontaneously (e.g., NZBWF1, NZM, BXSB, and MRL) are used as models of sensitive populations to determine if a specific drug or chemical exposure can affect the natural progression of disease. [c] 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. [d] Graft versus host disease. [e] Experimental autoimmune encephalomyelitis. [f] Tetramethylpentadecane.

Autoimunity a chemické látky

Table 2. Features of Autoimmunity Shared by Different Autoimmunity-Inducing Drugs and Chemicals

drugs/chemicals	mechanism	refs
silica/asbestos	adjuvant effect	*21*
	T regulatory cells	*33*
	inflammasome (NLRP3[a], IL-1β)	*28*
	exacerbates lupus-prone genotype	*32*
heavy metals (Hg, Ag, and Au)	T cell activation threshold	*86*
	T regulatory cells	*80*
	inflammasome (NLRP3[a], IL-1β)	Toomey and Pollard (unpublished)
	exacerbates lupus-prone genotype	*76, 108*
drugs (procainamide)	T cell activation threshold	*12*
	central tolerance	*18*
TCDD, dioxin[b]	central tolerance	*152*
	Ahr	*150*
	T regulatory cells	*150*
adjuvant oils (pristane)	adjuvant effect	*134*
	exacerbates lupus-prone genotype	*135*
pesticides/fungicides (HCB)	adjuvant effect	*162*
	exacerbates lupus-prone genotype	*164*
adulterated rapeseed oil	exacerbates lupus-prone genotype	*44*
iodine	T regulatory cells	*110*
TCE	exacerbates lupus-prone genotype	*153*

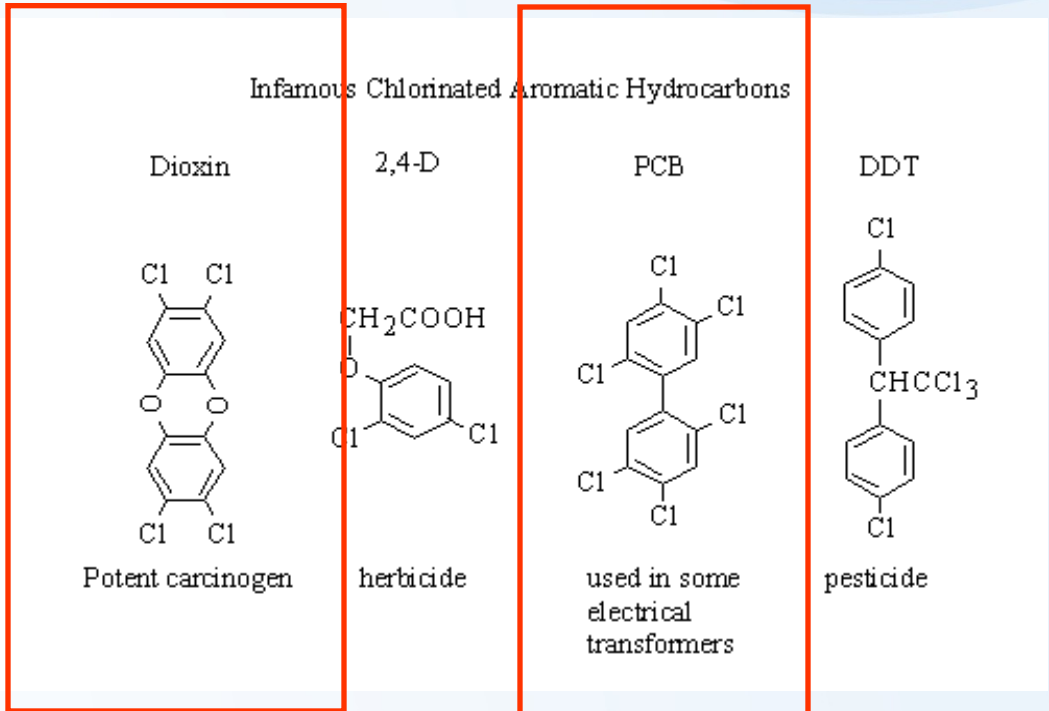
[a] NOD-like receptor family, pryin domain containing 3. [b] 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin.

Významné imunotoxické látky

Polychlorované bifenyly (PCBs)
Polychlorované dioxiny a furany (PCDD/Fs)

Persistentní organochlorové látky

- POPs - (stále) NEJVÝZNAMNĚJŠÍ ORGANICKÉ KONTAMINANTY ŽP:
  - odolávají degradaci, persistence v prostředí, bioakumulace
- Heterogenní skupina látek - zde diskuze PCDD/Fs a PCBs
  - **PCDD/Fs**
    - vedlejší produkty spalování
  - **Polychlorované bifenyly (PCBs)**
    - průmyslové chemikálie



Centrum pro výzkum toxických látek v prostředí

Co je známo in vivo – PCDD/Fs ?

- Imunotoxicita u lab. zvířat
  - NOEL 50 ng/kg (!!!!)
  - zvýšení infekcí Salmonella / E.coli
  - úmrtnost mláďat na Listeriozu
  - citlivost na viry / vyšší růst nádorů
- Imunotoxicita u primátů
  - NOEL 3 ng/kg - podobné účinky jako u hlodavců
- Epidemiologie - lidská populace
  - 1973 - kontam. masa PBBs (USA, Michigan)
  - 1979 - kontaminace oleje na Taiwanu (PCDF/PCB)
    - vzrůst kožních a respir. infekcí
    - pokles IgA a IgM a další efekty
- Imunosuprese u velkých vodních savců
  - tuleni, delfíni: hromadné úhyny na oportunní morbiliviry
  - akumulace PCBs: imunosuprese

POPs a neobvyklé mortality na virové infekce → imunosuprese

Environmental Pollution
Volume 152, Issue 2, March 2008, Pages 431-442

doi:10.1016/j.envpol.2007.06.075 | How to Cite or Link Using DOI

Permissions & Reprints

Cited By in Scopus (3)

Persistent organic pollutants (POPs) in Caspian seals of unusual mortality event during 2000 and 2001

Natsuko Kajiwara[a], [1], Mafumi Watanabe[a, 1], Susan Wilson[b], Tariel Eybatov[c], Igor V. Mitrofanov[d], David G. Aubrey[e], Lev S. Khuraskin[f], Nobuyuki Miyazaki[g] and Shinsuke Tanabe[a]

Centrum pro výzkum toxických látek v prostředí

The Risk of Infection from Polychlorinated Biphenyl Exposure in the Harbor Porpoise (*Phocoena phocoena*): A Case–Control Approach

Ailsa J. Hall,[1] Kelly Hugunin,[2] Robert Deaville,[3] Robin J. Law,[4] Colin R. Allchin,[4] and Paul D. Jepson[3]

[1]Sea Mammal Research Unit, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Fife, United Kingdom; [2]North Carolina State College of Veterinary Medicine, Raleigh, North Carolina, USA; [3]Institute of Zoology, Zoological Society of London, London, United Kingdom; [4]Centre for Environment, Fisheries and Aquaculture Science, Burnham Laboratory, Burnham-on-Crouch, Essex, United Kingdom

Environ Health Perspect 114:704–711 (2006).

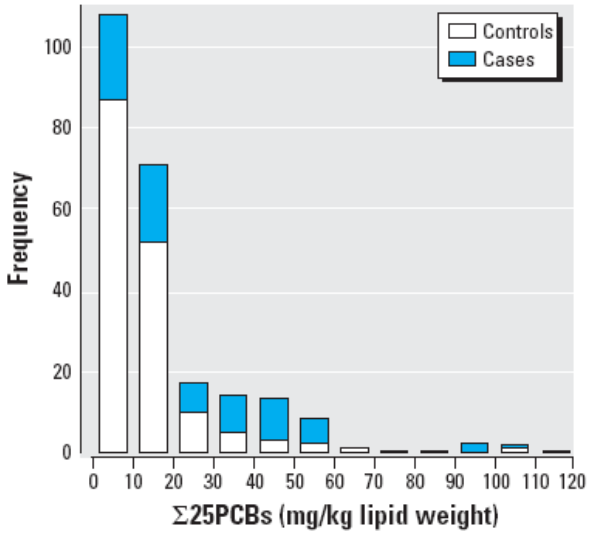


Figure 1. Frequency distribution of Σ25PCBs in the blubber of harbor porpoises selected as cases or controls.

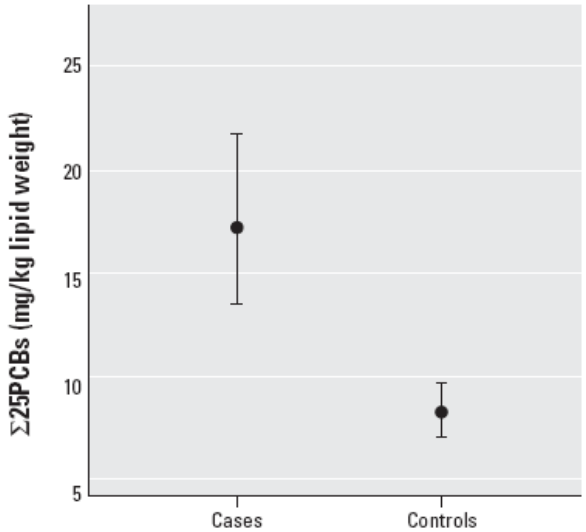


Figure 2. Geometric mean Σ25PCBs (geometric 95% CI) in the blubber of harbor porpoises for cases and controls.

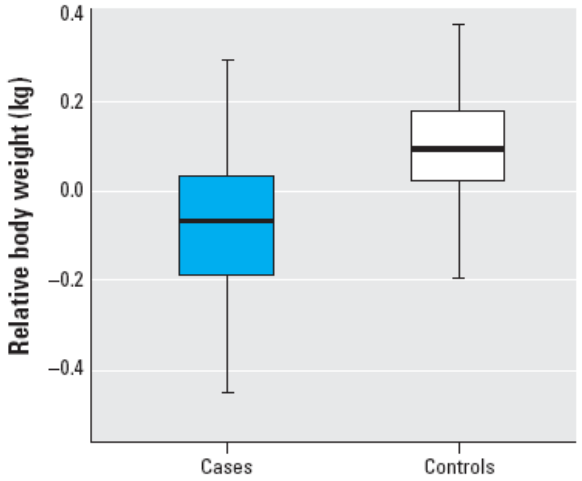


Figure 3. Relative body weight of harbor porpoises [residuals around the best-fit linear regression between ln(body mass) and ln(body length)] among the cases and controls. Values shown are median, 25th–75th percentiles, and minimum–maximum.

Centrum pro výzkum toxických látek v prostředí

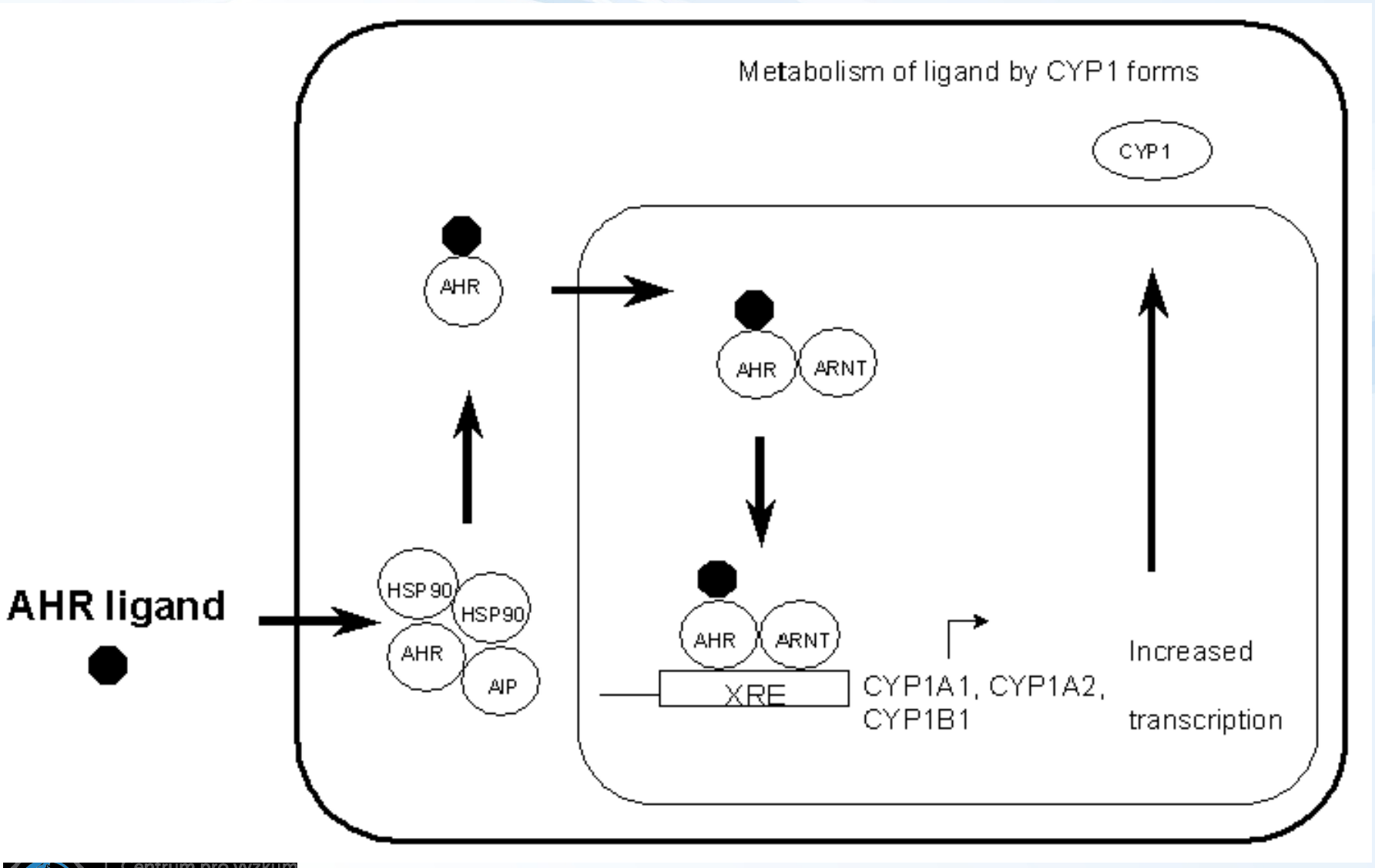
Hlavní mechanismus působení – planárních POPs → AhR

- **AhR - Arylhydrocarbon receptor**
  (Receptor pro planární aromatické uhlovodíky)
  - intracelulární transkripční faktor
  - příbuzný ostatním „nukleárním" receptorům (receptory pro nízkomk. Hormony: ER, AR, ThR, RAR/RXR …)

- **Přirozená funkce (?) - reakce na toxické látky**
  → syntéza detoxikačních enzymů (CYP450)

- **Aktivace AhR v přítomnosti PCDD/Fs, PCBs**
  - MNOHO různých vedlejších toxických účinků
  - (např. karcinogenita…) + také IMUNOTOXICITA

Mechanismus signálování AhR



AhR v Imunitním systému

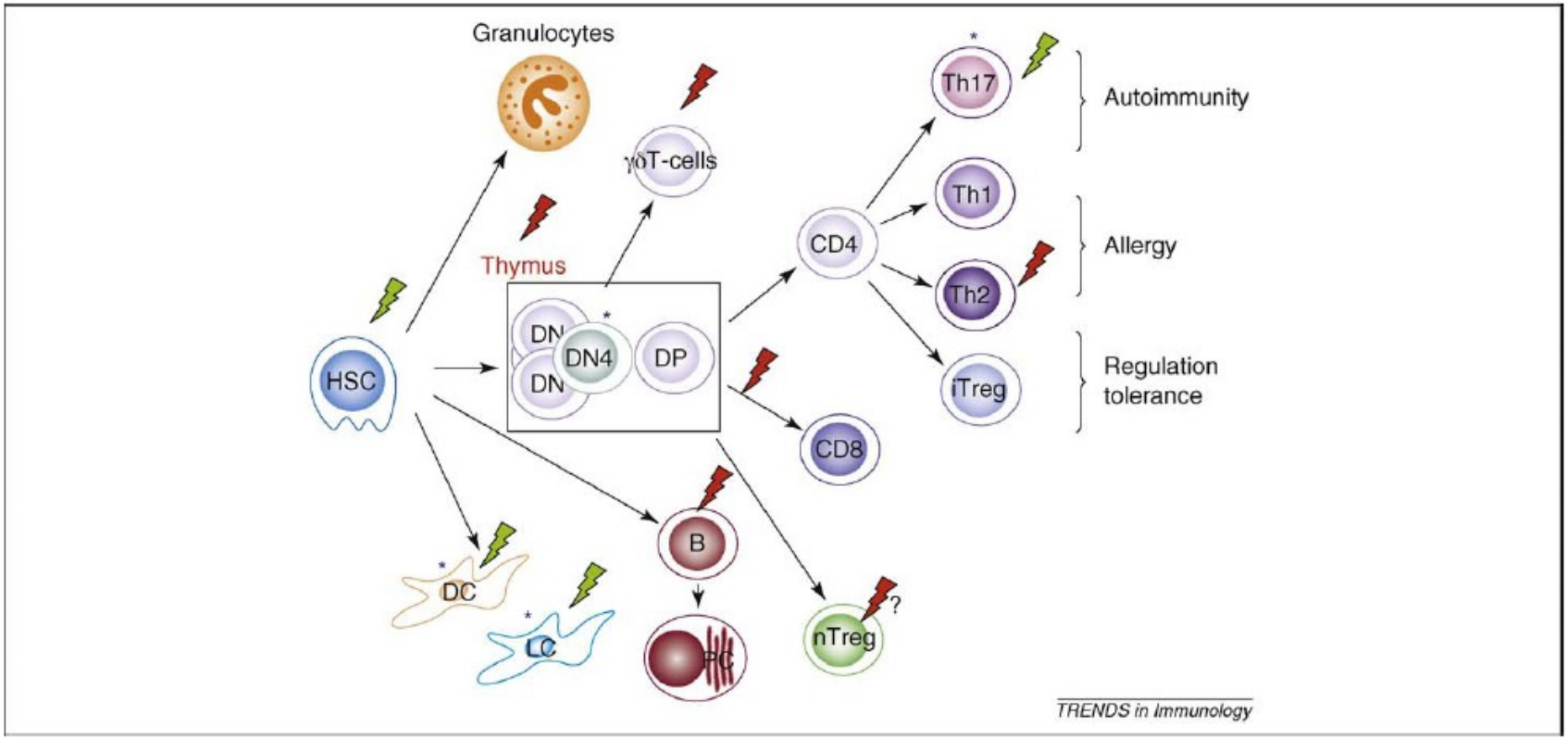


Figure 1. Schematic compilation of current evidence of the influence of AhR on immune function and differentiation. The major lymphoid and myeloid differentiation lines are shown. The red flashes indicate cells where AhR overactivation by *in vivo* or *ex vivo* exposure to dioxin has been reported to result in immunotoxic effects (e.g. biased differentiation, lack of cytokine secretion, or altered function). It is important to note that many of these effects are presumably indirect effects because some of the cells affected express little or no AhR. Blue asterisks indicate cells for which AhR expression has been experimentally confirmed by either RNA measurements or Western blotting. Green flashes indicate evidence of a direct physiological role for AhR (e.g. necessary for cell-specific expression of characteristic genes, involved in maturation or differentiation). DC, dendritic cells; DN, double negative thymocyte; DP, double positive thymocyte; HSC, hematopoietic stem cell; LC, Langerhans cell; nTreg, natural T regulatory cell; PC, plasma cell; iTreg, induced T regulatory cell.

AhR v Imunitním systému

Box 3.

Environmental links of AhR to autoimmune diseases

Epidemiology, anecdotal evidence and mechanistic studies suggest links between autoimmune diseases and environmental exposure to small chemicals and/or AhR ligands or xenobiotic-metabolizing enzyme activity. Such links include:

- Dioxin and rheumatoid arthritis [69]
- Smoking and rheumatoid arthritis [70]
- Smoking and psoriasis [72]
- UV light and systemic lupus erythematosus [71]
- Cytochrome P450 RNA levels and multiple sclerosis [73]

Table 1. Some clinically or quantitatively relevant ligands of AhR[a].

Endogenous
FICZ, 6-formylindolo[3,2-*b*]carbazole (tryptophan photoproduct)
Bilirubin (product of heme metabolism by the liver)
Lipoxin A4 (eicosanoid with anti-inflammatory properties)
ITE [2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester] (isolated from lung tissues)
Environmental pollutants (formed during combustion of organic material)
2,3,7,8-tetrachlorodibenzo-*p*-dioxin
Benz[*a*]pyrene
Dietary
Quercetin (present in apples and onions)
Indol-3-carbinol (present in many Brassicaceae, e.g. cabbage)
Resveratrol (present in red wine)
Curcumin (a spice frequently used in Indian cuisine)
Drugs (synthetic)
M50367 {3-[2-(2-phenylethyl) benzoimidazole-4-yl]-3-hydroxypropanoic acid}
VAF347 {[4-(3-chloro-phenyl)-pyrimidin-2-yl]}

[a]For comprehensive lists and a discussion of immunological relevance see Refs [4] and [23].

Centrum pro výzkum toxických látek v prostředí

Role AhR v autoimunitách – diferenciace Th17

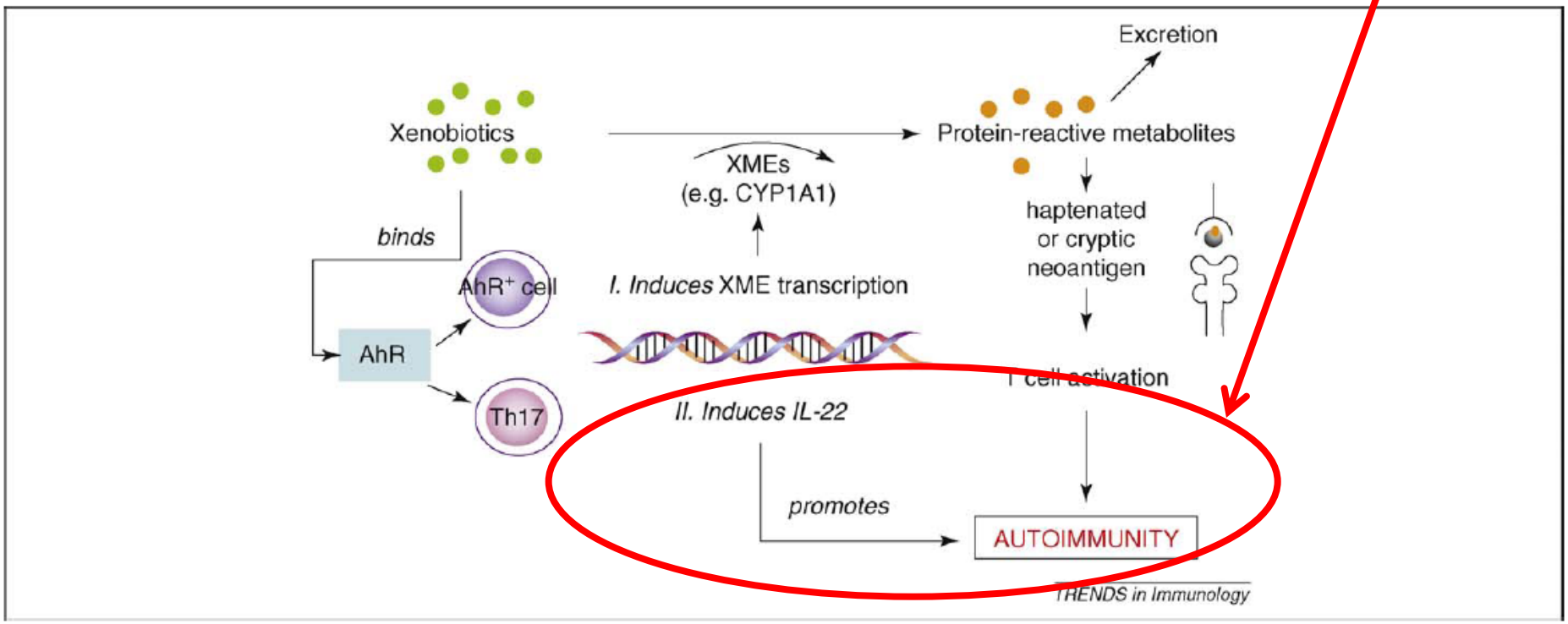
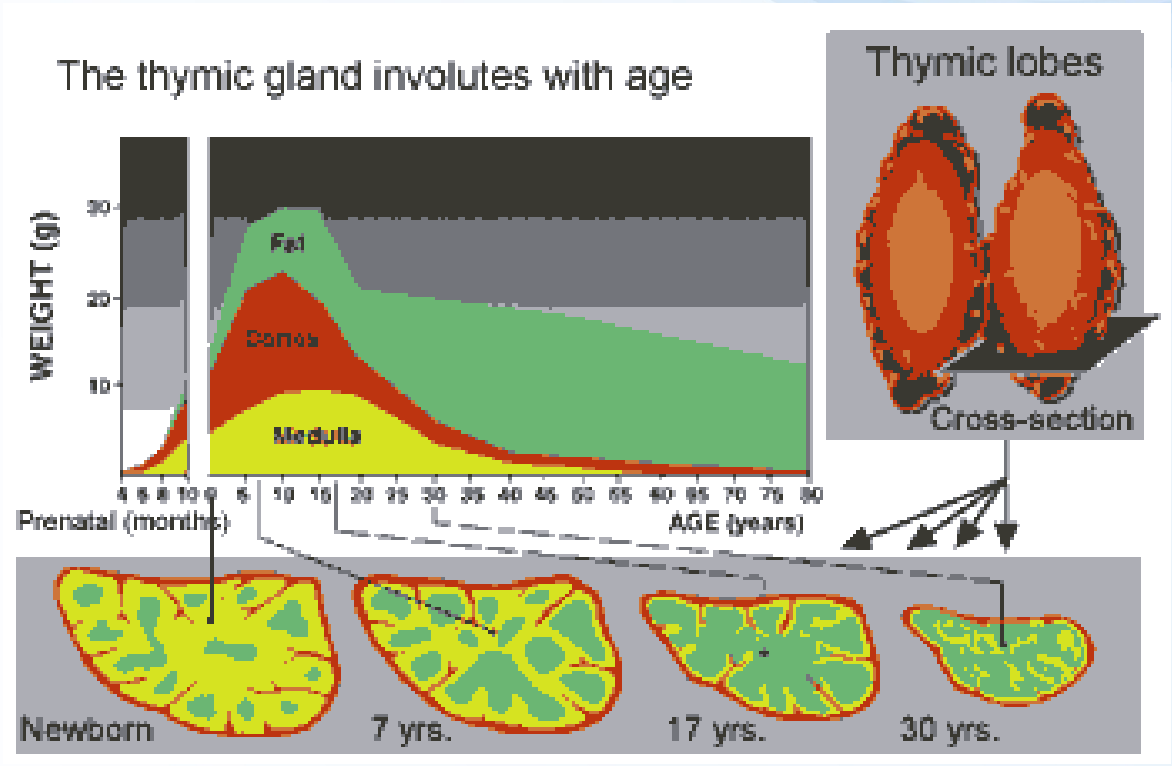


Figure 2. Two pathways by which direct gene induction via AhR could contribute to initiation and/or exacerbation of autoimmune diseases. (I) Low-molecular-weight chemicals and in particular their more reactive metabolites – formation of which is under control of AhR-inducible phase I and phase II metabolizing enzymes – (xenobiotic metabolizing enzymes, XMEs) can form protein adducts, leading to the formation of new self antigens against which no tolerance exists. For instance, the phenotype of slow acetylation by N-acetyltransferase, a phase II enzyme, is more common in patients with drug-induced lupus caused by procainamide or hydralazine [67]. Variant alleles of *CYP1A1*, which code for enzymes with higher activity, might protect against psoriasis [68]. The links between dioxin and rheumatoid arthritis (RA) and between psoriasis and smoking are strong. Smoke contains numerous strong AhR ligands, including benz[*a*]pyrene dioxin and other planar aromatic hydrocarbons [69–71]. (II) AhR can mediate induction of cytokine genes in a cell-specific manner. AhR is necessary for IL-22 secretion by Th17 cells. Where IL-22 is relevant in autoimmune disease, this might exacerbate effects. An example is the skin, where IL-22 involvement in psoriasis is known. However, it should be noted that IL-22 has other functions; in particular, it is essential in protection against infectious agents.

Imunotoxické efekty spojené s aktivací AhR

1) prenatální toxicita pro vývoj brzlíku

- AhR je exprimován ve vysokých koncentracích např. v játrech (detoxifikace), ale také v brzlíku (význam není zcela jasný)
- působení PCBs, PCDDs → apoptoza
  - → **PRENATÁLNÍ ATROFIE THYMU** (urychlení přirozené konvoluce)
  - → T-buňky nemohou dozrát



Centrum pro výzkum toxických látek v prostředí

Imunotoxické efekty spojené s aktivací AhR

2) Imunotoxicita PCDD/Fs, PCBs v dospělosti

- **V dospělosti - AhR menší význam**
  - imunotoxicita u myší s i bez AhR
    (experimenty s AhR knock-out kmeny)

- ALE: zvýšená citlivost v přítomnosti AhR
  - př. Suprese Ab proti SRBC

- Prokázány především účinky **na B-bb. a protilátkovou odpověď**

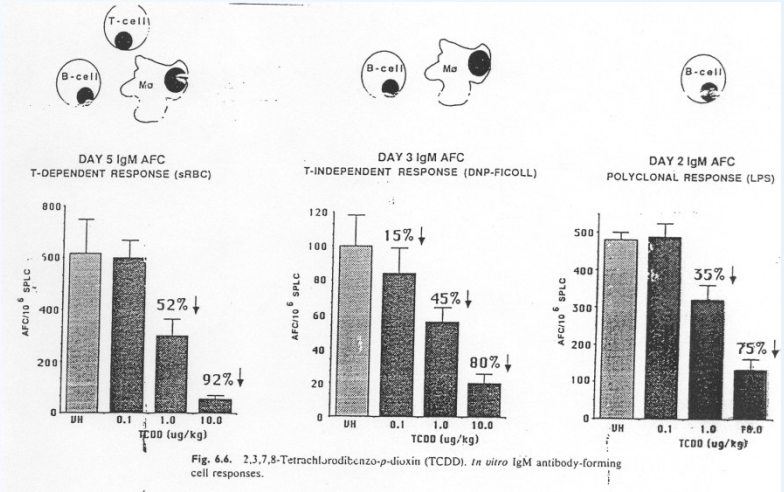


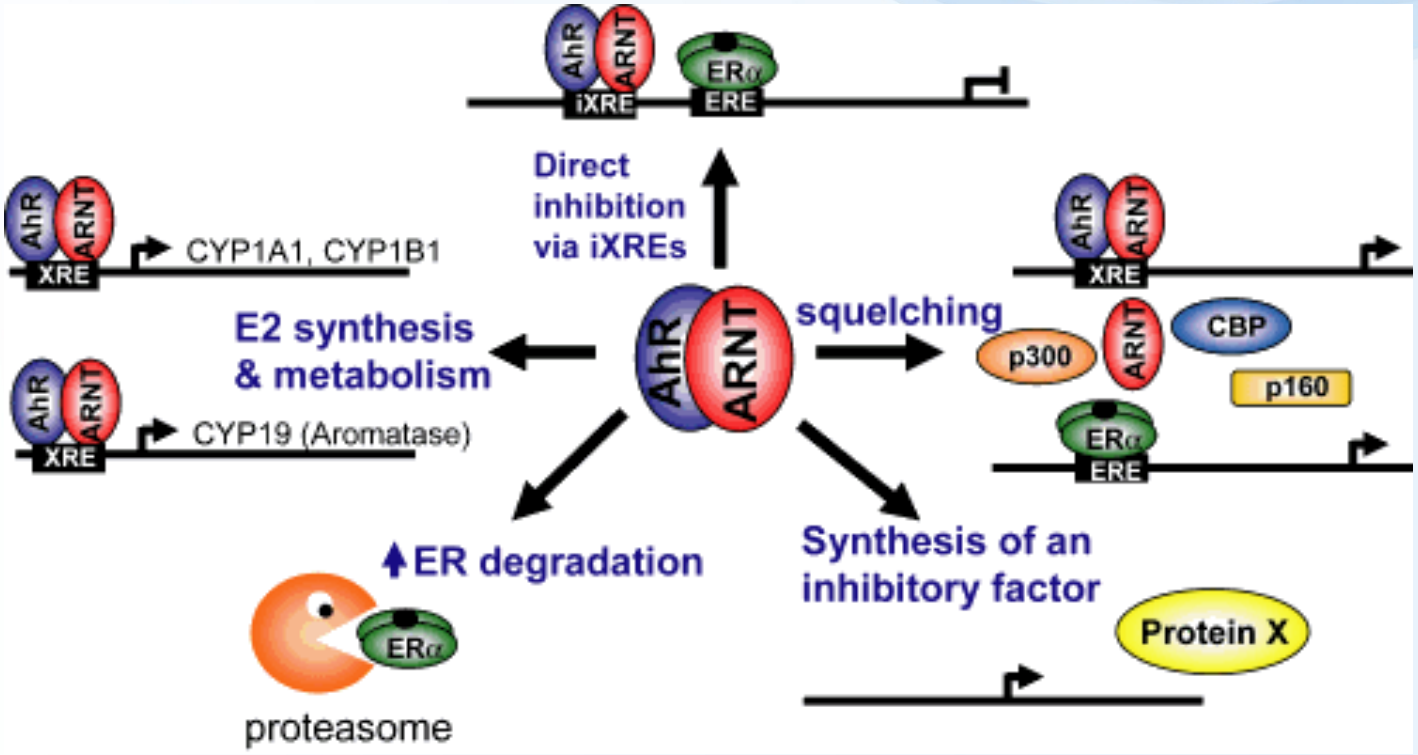
Fig. 6.6. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). *In vitro* IgM antibody-forming cell responses.

Pro připomenutí …
TCDD – toxicita zejména pro B-buňky

Nepřímá toxicita PCDD/Fs a PCBs

- **Působení na edokrinní systém**
  - glukokortikoidy, steroidní h., thyroxin…
    - vzrůst sérového kortikosteronu *(obecně: marker stresu)* → imunosuprese
  - Vzrůst nádorů u myší po působení PCBs (M > F)
  - Cross-talk mezi receptory (AhR vs. ER …)

Cross-talk
AhR vs. ER



Centrum pro výzkum toxických látek v prostředí