IMMUNOTOXICOLOGIE

Master Biochimie-Immunologie

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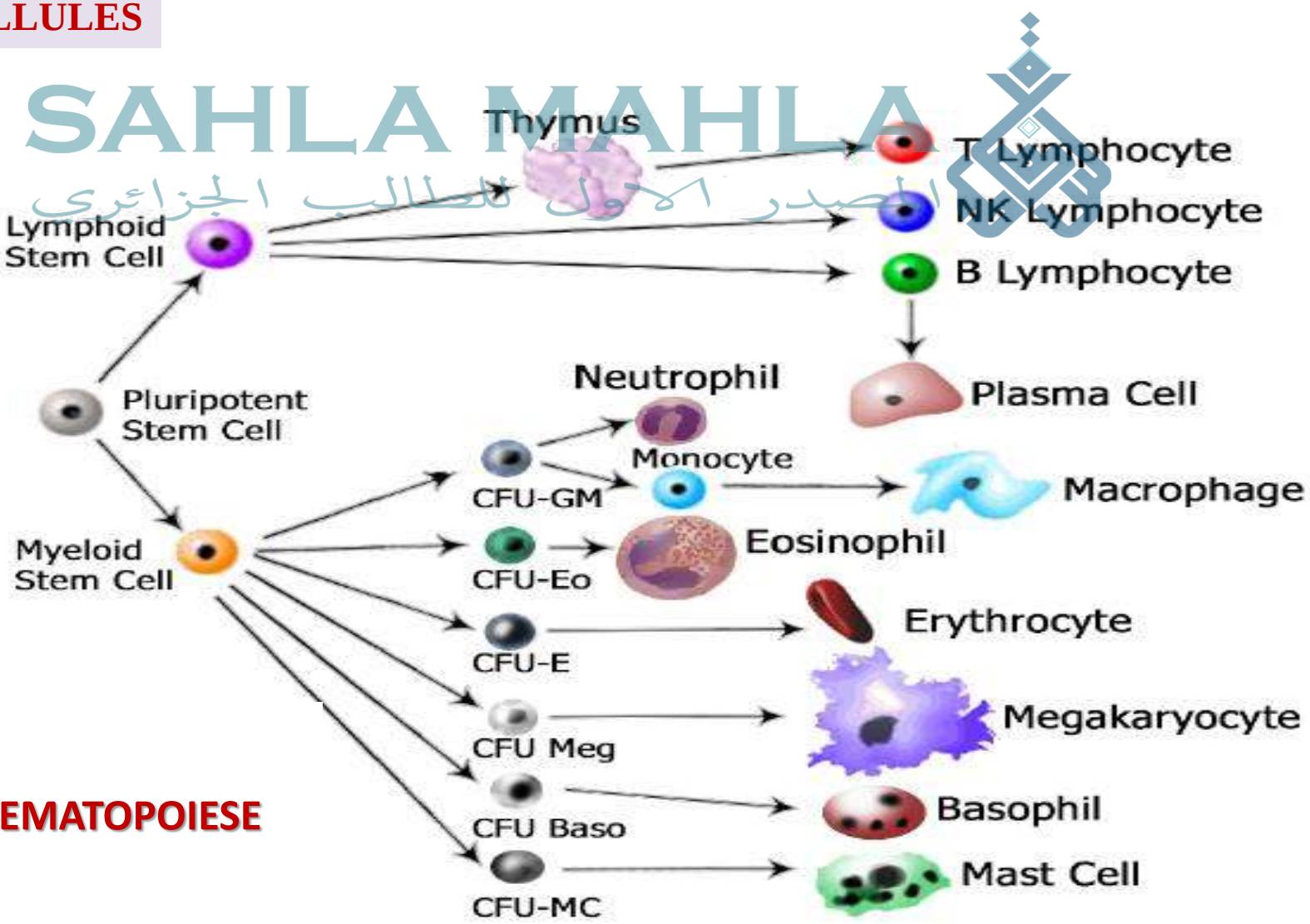
SAHLA MAHLA

المصدر الاول للطالب الجزائري

Organisation du Système Immunitaire

CELLULES



HEMATOPOIESE

Marqueurs des lymphocytes B

- BCR
- CD19, CD20
- CD79a/CD79b
- CD22, CD72
- CD40
- CD80/86

- Récepteur de l'Ag
- Marqueurs de lignée B
- Transduction du signal
- Molécules d'adhésion
- Co-activation /CD4OL
- Co-activation /CD28

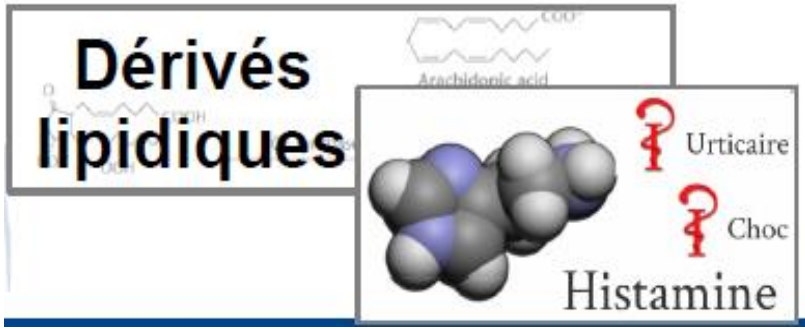
Marqueurs des lymphocytes T

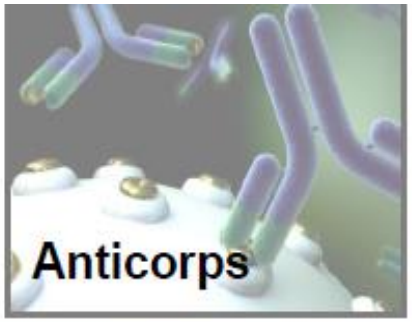
- TCR
- CD2, CD5, CD7
- CD3
- CD4
- CD8
- CD28
- CD40L
- CD25

- Récepteur de l'Ag
- Marqueurs de lignée T
- Transduction du signal
- Liaison MHC II
- Liaison MHC I
- Co-activation/B7
- Co-activation CD40
- Récepteur à l'IL2

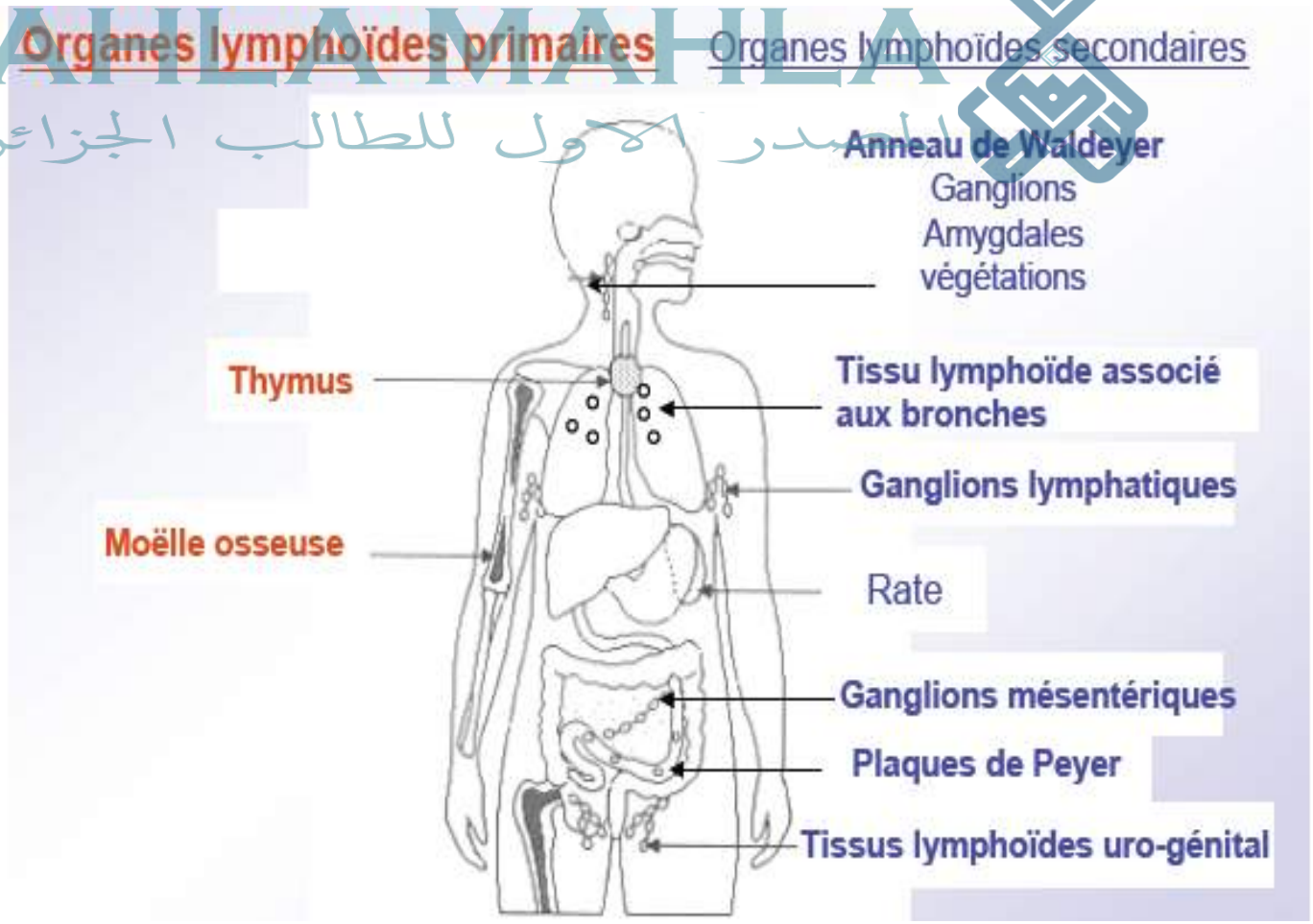
MOLECULES

Cytokines

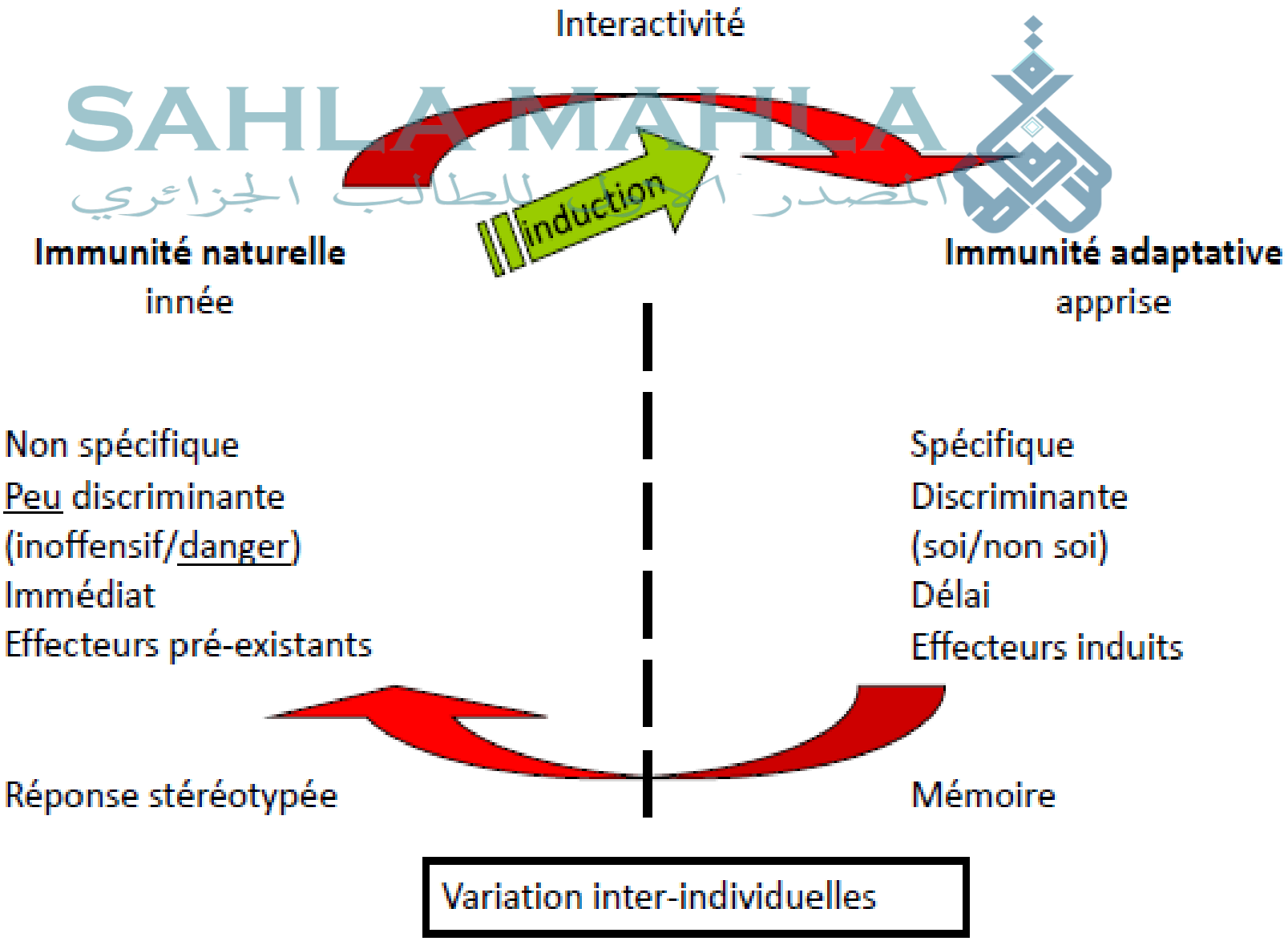




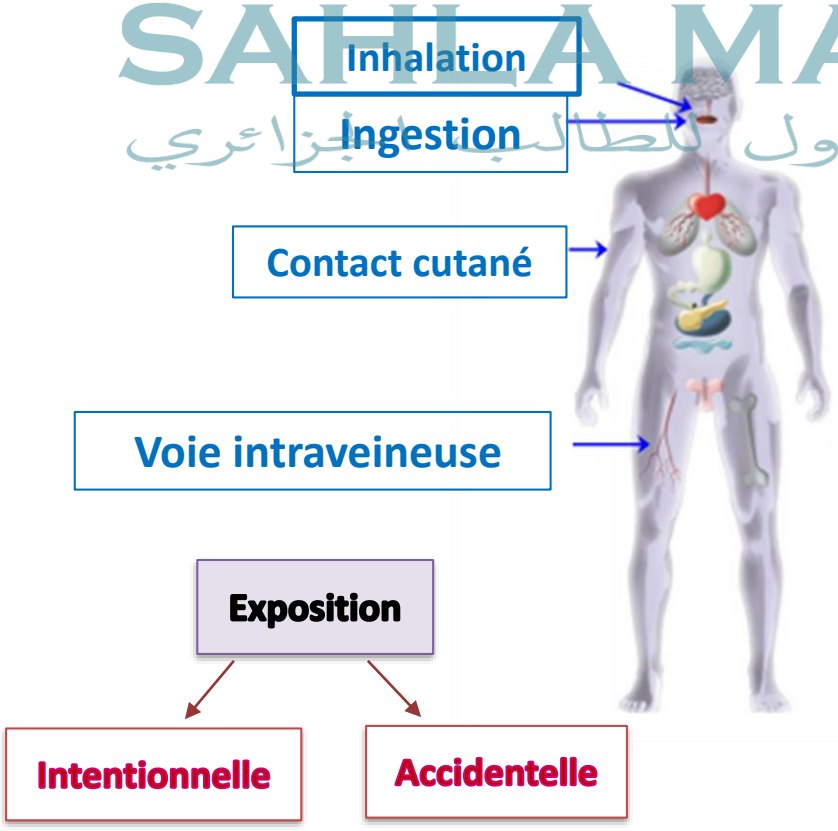
ORGANES



LES RÉPONSES IMMUNITAIRES



LES XÉNOBIOTIQUES



*Activation:

Désirable:

- ✓ Vaccins
- ✓ Effets anti-tumoraux

Indésirable:

- ➢ Inflammation
- ➢ Anaphylaxie
- ➢ Hypersensibilité

*Suppression:

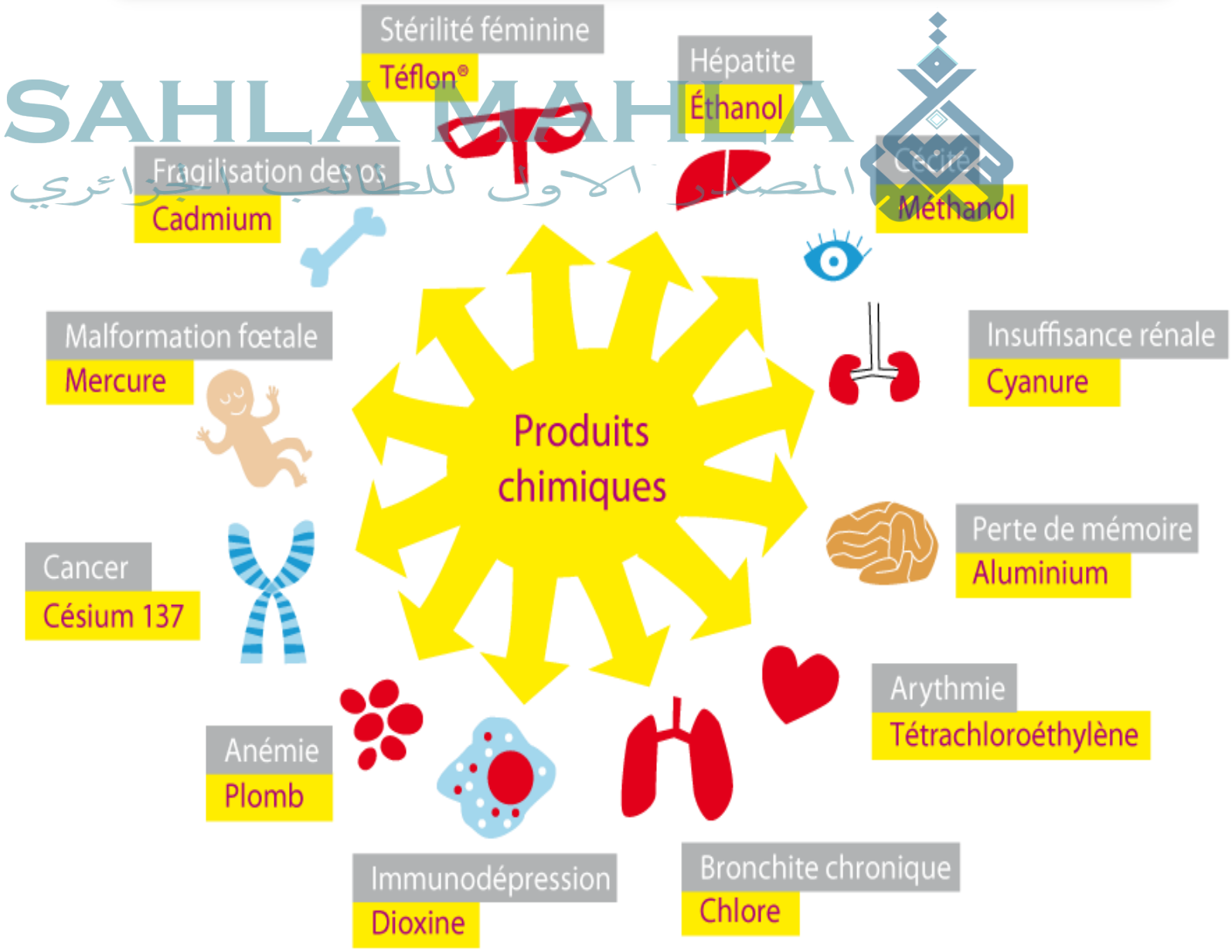
Désirable:

- ❖ Traitement des troubles inflammatoires
- ❖ Prévention des réponses allergiques
- ❖ Non rejet des transplantations

Indésirable:

- ➢ Réponse réduite à l'infection et au cancer
- ➢ Myélosuppression et dysfonction thymique

Toxicité des produits chimiques sur l'organisme



Principales classes d'agents immunotoxiques

Les substances chimiques

Pesticides, Additifs, Colorants, Hydrocarbures, Polycycliques aromatiques, tabac……

Les allergènes

Acariens, pollens, moisissures, aspartam, oeuf, lait, médicaments, latex……

Les médicaments agissant sur le SI

Toxicité des produits chimiques sur le système immunitaire

Chemical class	Example	Laboratory immune abnormality
Polyhalogenated aromatic hydrocarbons	TCCD	+
	PCB	+
	PBB	+
	HCB	+
Heavy metals	Lead	+
	Cadmium	+
	Methyl mercury	+
Aromatic hydrocarbons (solvents)	Benzene	+
	Toluene	+
Polycyclic aromatic hydrocarbons	DMBA	+
	BaP	+
	MCA	+
Pesticides	O,O,S-TMP	+
	Carbofuran	+
	Chlordane	+
Organotins	DOTC	+
	DBTC	+
Aromatic amines	Benzidine	+
Oxidant gases (air pollutants)	NO_2	+
	O_3	+
	SO_2	+
Others	Asbestos	+
	DMN	+

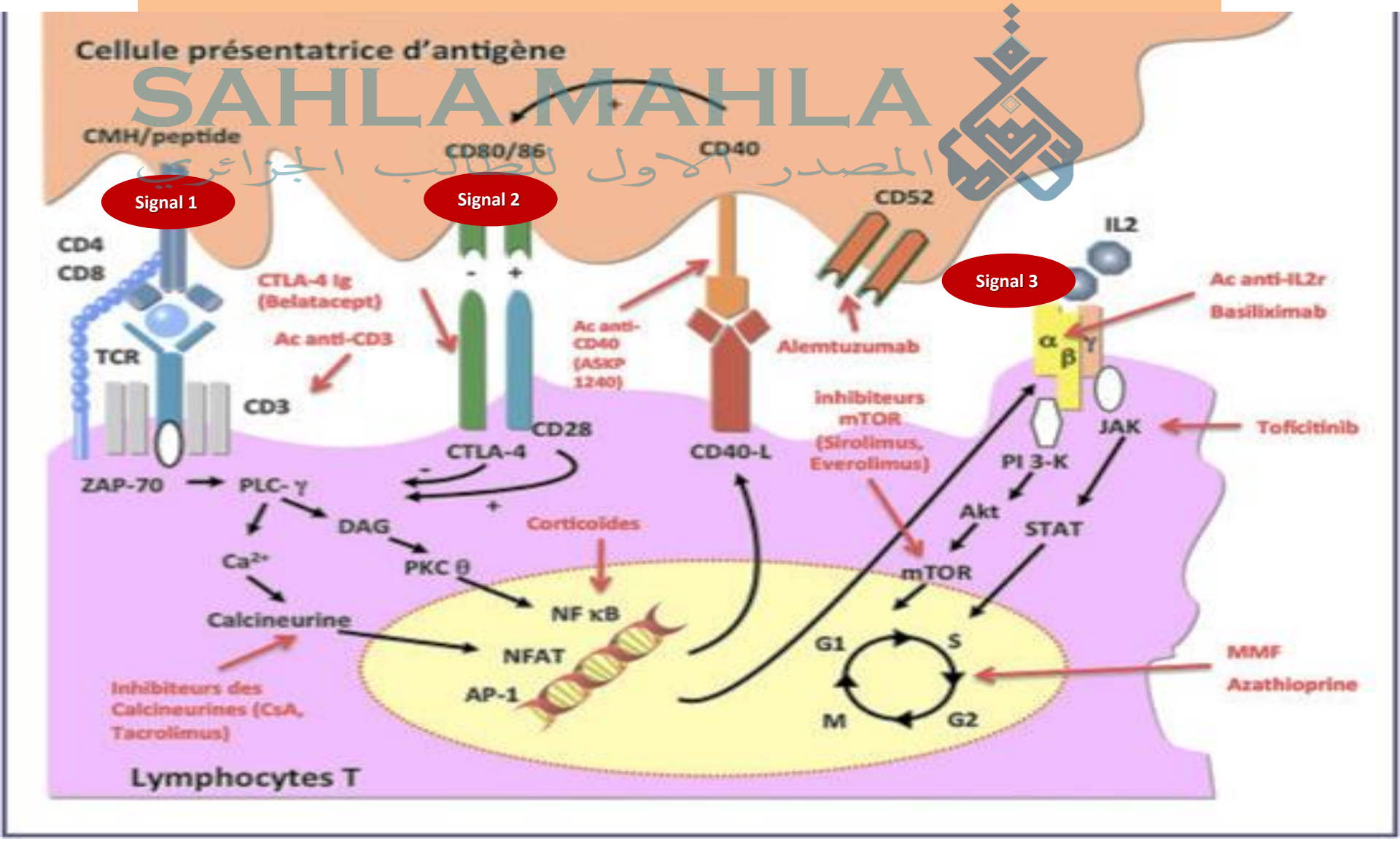
Médicaments agissant sur le SI

-Immunomodulateurs: modifient la réponse immunitaire de l'organisme sans majorer de risque infectieux ou tumoral

-Immunostimulants: renforcent les capacités de défense de l'organisme

-Immunosupresseurs: Altèrent les capacités de l'organisme à développer une réaction immunitaire

Sites d'action des immunosuppresseurs



Exemples du mode d actions de quelques médicaments immunosuppresseurs

Inhibition du signal 1

a- inhibiteurs de la calcineurine

Cyclosporine

b- Agents biologiques (Anticorps)

Muromonab CD3 (Orthoclone OKT3):

c- Glucorticoïdes

Inhibition du signal 2

Déxaméthasone

d- Agents antiproliferatifs et antimétabolites

Inhibition du signal 3

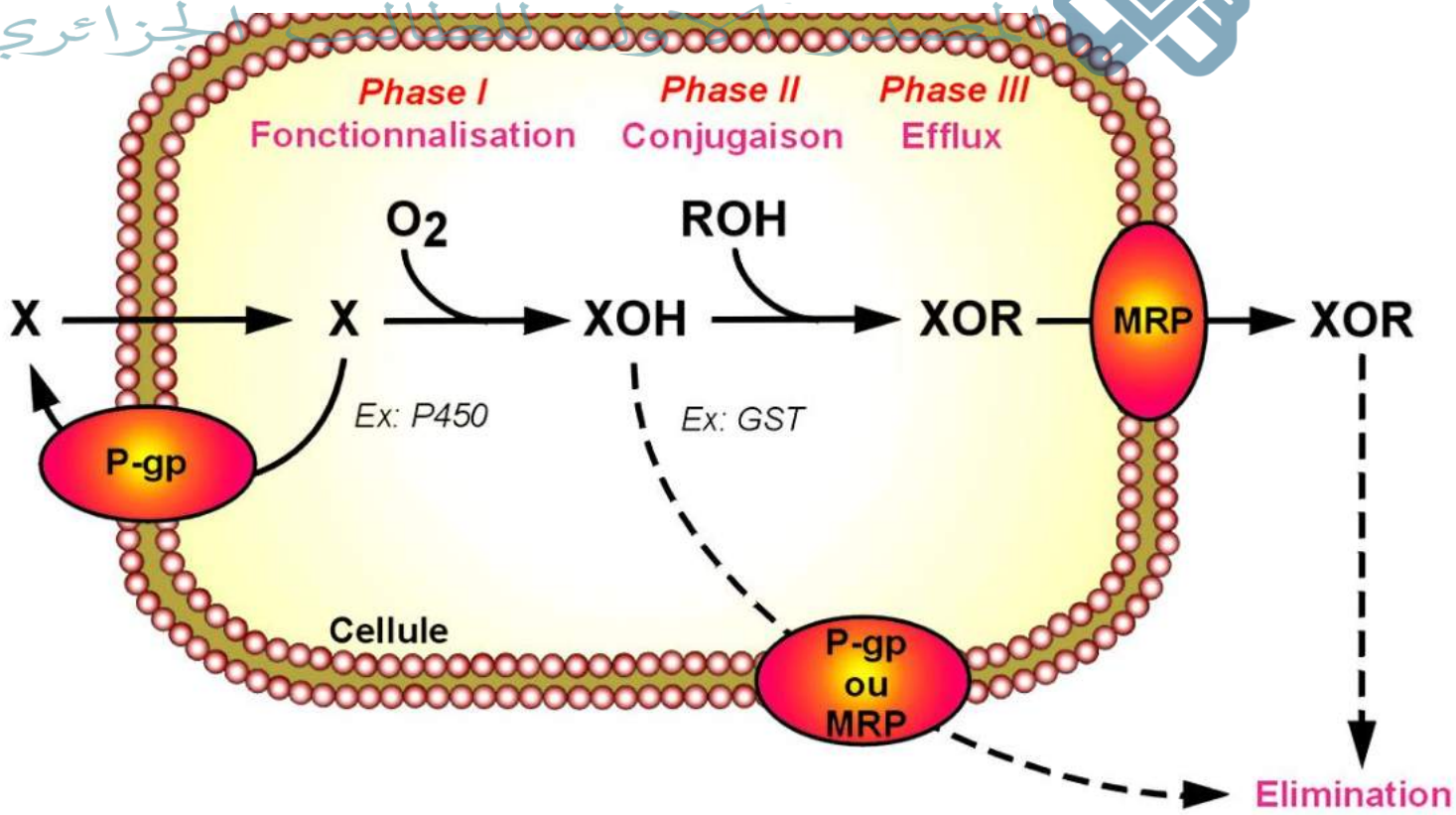
- Agents antiproliferatifs

Cirolimus

-Agents antimétabolites

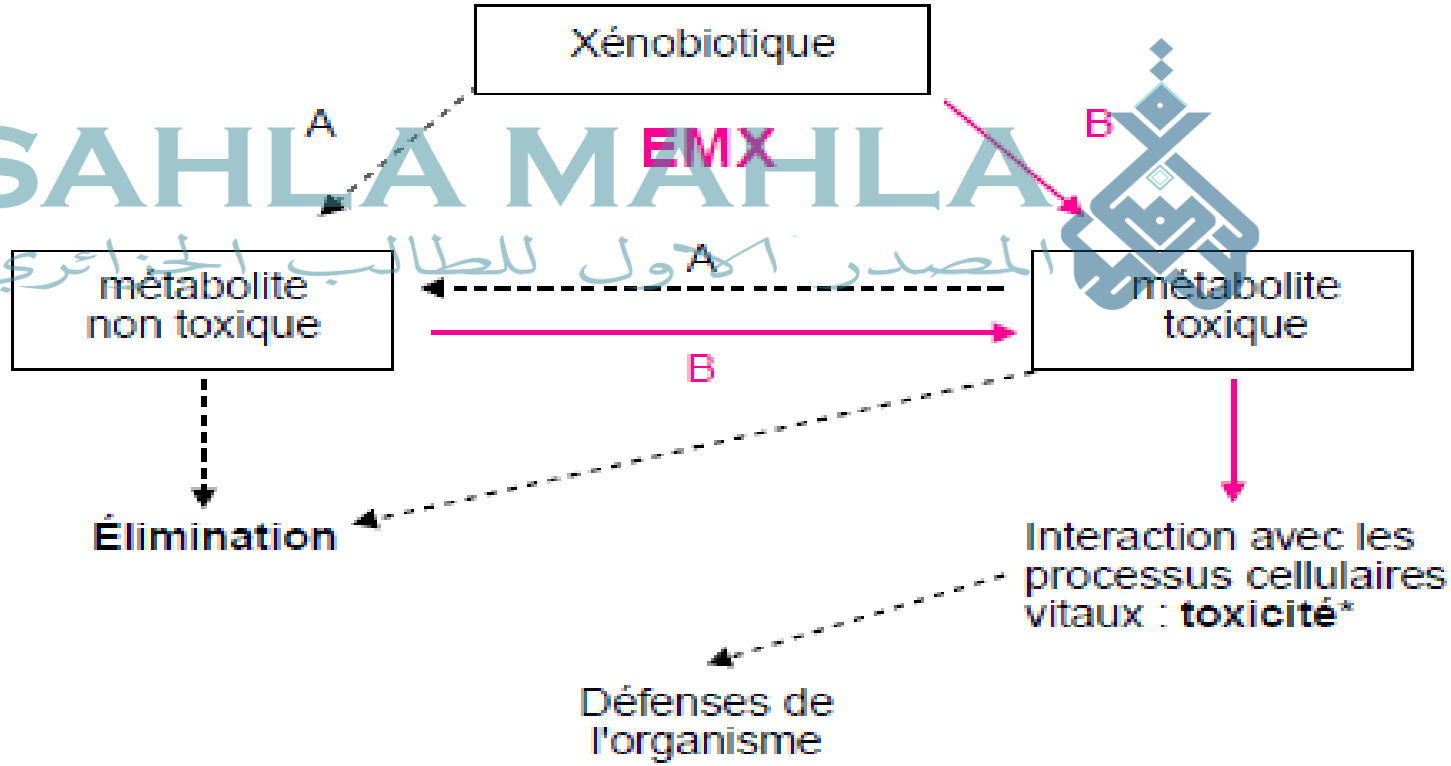
Azathioprine

Métabolisme des xénobiotiques



ENZYMES DE PHASE I	
OXYDASES	**REDUCTASES**
Cytochromes P450	Cytochromes P450
Monooxygénases à flavine	Glutathion peroxydases
Peroxydases	Carbonylréductases
Xanthine oxydases	Alcool déshydrogénases
Monoamine oxydases	**HYDROLASES**
Alcool déshydrogénases	Estérases
Aldéhyde déshydrogénases	Epoxyde hydrolases
ENZYMES DE PHASE II	
UDP-glucuronyltransférases	O-, N-, S-méthyltransférases
Glutathion-S-transférases	N-acétyltransférases
Sulfotransférases	
PROTEINES DE PHASE III	
P-glycoprotéine	OATP, protéines de transport des anions organiques
MRP, protéines de résistance multi-drogue	
BCRP, protéines de résistance dans le cancer du sein	OCT, transporteur de cations organiques

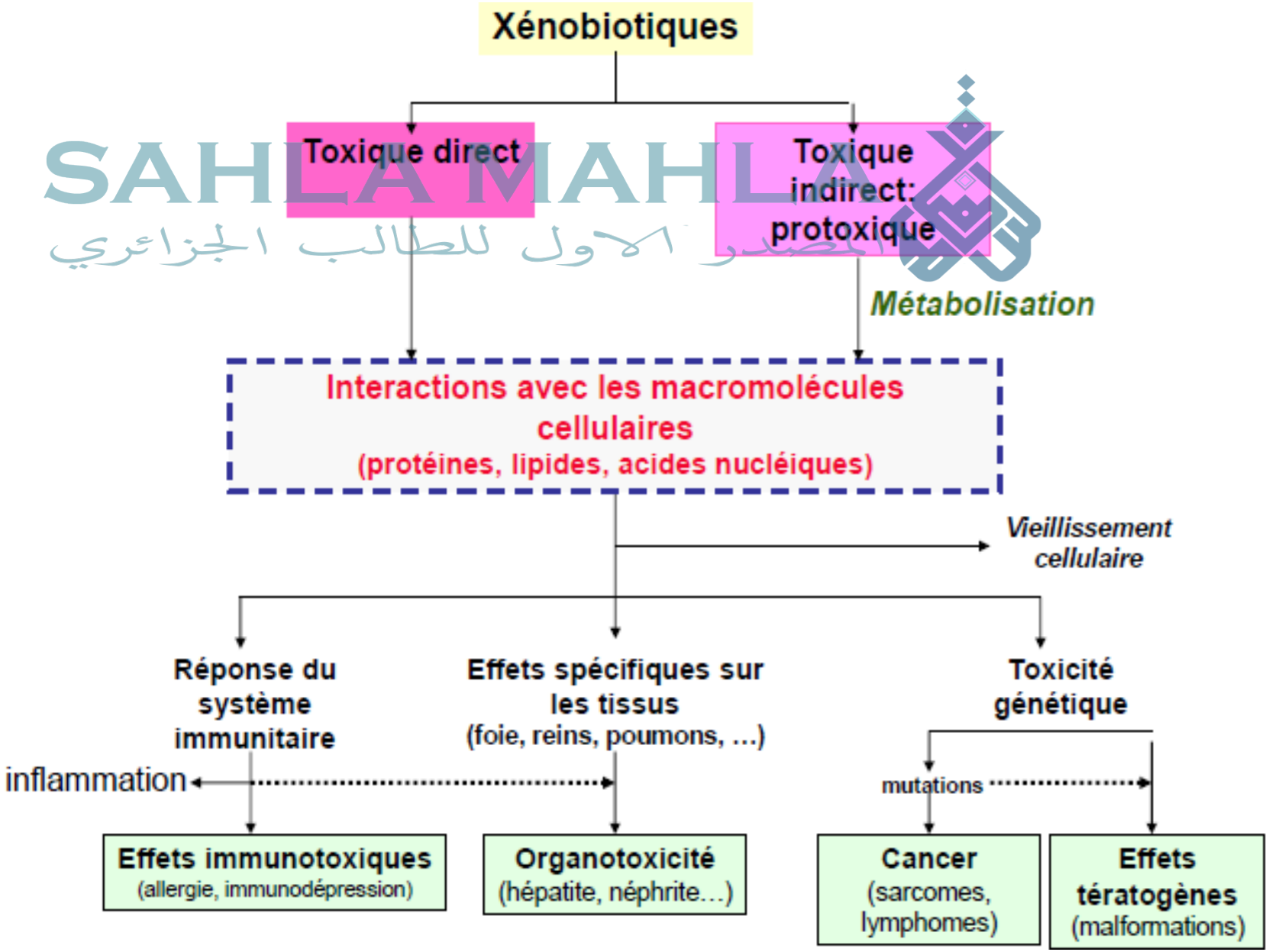
Exemples d'enzymes participant au métabolisme des xénobiotiques



* La toxicité dépend, entre autres, de l'équilibre entre les voies de :
- toxication B →
- détoxication A - - ►

L'expression des EMX dépend de facteurs : - génétiques
- environnementaux
- physiopathologiques

Equilibre toxication/détoxication



Effets immunotoxiques causés par les hydrocarbures polycycliques aromatiques (HPA)

S.N.	Name of Compounds	Immune Responses
A.	Polycyclic Aromatic Hydrocarbons (PAHs)	▪ Impairment of humoral and cell-mediated immune function. ▪ Immunosuppressive agent.
1.	Benzo[a]pyrene	▪ Immunosuppressant and immunotoxicants. ▪ Diminish humoral immunity, increases T cell mitogenic activity. ▪ Induce pro-inflammatory cytokines production (IL-1β, iNOS). ▪ Inhibited the up-regulation of markers such as CD1a, CD80 and CD40 found in dendritic cells during monocyte differentiation into dendritic cells upon the action of GM-CSF and IL-4. ▪ Modulate Vitamin-D3 signaling via activating Aryl hydrocarbon receptor. ▪ Increases IL-4 mRNA expression.
2.	7,12-Dimethyl benzo [a]anthracene (DMBA) **Article 3**	▪ Suppress splenocytes proliferation up to 90% during mitogen and alloantigen-induction. ▪ Repression of in vitro humoral immune response of murine splenocytes.
3.	Benz[a]anthracene	▪ Induces oxidative DNA damage in lymphocyte.
4.	3,6-bis(2 piperidinoethoxy) acridinetrihydrochlorid	▪ Immunomodulator, enhances cytotoxic activity of Natural killer cells.

Effets immunotoxiques causés par les pesticides

S.N.	Name of Compounds	Immunological responses
1.	Organophosphate	
	Chloropyrifos	▪ Increases CD26 expression on cytotoxic T cells. ▪ Decreases CD15 cells production (Autoantibody producing B cells). ▪ Reduces mitogenesis immune cells in response to concanavillin and phytohemagglutinin. ▪ Amplifies autoantibodies production.
	Diisopropyl methyl phosphonate	▪ Inhibits activity of human and murine NK cells and murine cytotoxic T cells.
	Malathion	▪ Suppresses of NO production and LPS-induced TNF-alpha generation. ▪ Increases antibody production following immunization with a T-lymphocyte dependent antigen and macrophage function and led to mast cell degranulation.
2.	Carbamate	
	Aldicarb	▪ Increases T8 lymphocytes population and decreases T4:T8 cell ratio. ▪ Increases lymphocytes proliferation and alters macrophage functions by decreasing with IL-1 production.
	Dimethoate	▪ Decreases total serum IgG and IgM and T-cells in the thymus, forming autologous rosettes.
	Sodium methyl dithiocarbamate	▪ Inhibits expression of IL-1α, IL-1β, IL-18, IL-12, IFN-γ, p35, p40 m-RNA level and macrophage migration inhibitory factor (MIF) whereas increases IL-10 m-RNA level.
	Mancozeb	▪ NO production decreases with the in vitro exposure. Suppresses TNF-γ secretion in vitro where as enhance release detected in ex-vivo experiment.
	Carbendazim	▪ Decreases B lymphocytes proliferation and serum IgG, IgM and IgA levels.
3.	Organochlorine	
	Endosulfan	▪ Decreases IgA and IgG production.
	Methoxychlor	▪ Decreases in IgM splenic plaque-forming cell responses, splenic T-cell (CD3+) populations and germinal center (GC) B-cell (CD19+PNA+) populations.
	Hexachlorocyclohexane	▪ Increases population of CD3 (+) CD4 (+) T-lymphocytes and expression of CD45RO (+) on CD4 (+) and CD8 (+) T-lymphocytes. ▪ Decreases CD4(+) CD25(+) T-lymphocytes and level of IL-2 and IFN-γ in SLE patients.
	o,p'-Dichlorodiphenyl -trichloroethane (DDT)	▪ Increases the percentages of CD3(+)CD4(+) T-lymphocytes and IL-10 level. ▪ Decreases CD4(+) CD25(+) T-lymphocytes and level of IL-2 and IFN-γ in SLE patients.

Effets immunotoxiques causés par les métaux lourds

S.N.	Name of Metal	Immunological responses
1.	Cadmium	▪ Increases production of NO. ▪ Inhibits synthesis of IgE by B cells or PBMCs upon IL-4/aCD40 stimulation and decreases proliferation of B cells or PBMCs. ▪ Increase production of chemoattractant Leukotriene B4 from neutrophils and monocytes.
2.	Lead (Pb)	▪ Decreases NO production in cytokine-induced cell lines. ▪ Induces production of TNF, IL-6 and IL-12 and decreases IL-10 production.
3.	Mercury (Inorganic)	▪ Increases IL-12, IL-17, IFN-γ and TNF-α production. ▪ Overexpression of CD86 and HLA-DR and production of TNF and IL-8 in vitro.
4.	Arsenic	▪ Decreases IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ secretion from T cells. ▪ Increases IgG, IgA and IgE level.

MÉTHODES D'ÉTUDE DE L'IMMUNOTOXICITÉ

Tests de routine

Tests d'immunosuppression

Non spécifiques

- ✓ résistance aux infections
- ✓ élimination de tumeurs

Spécifiques

Tests d'immunostimulation

Non spécifiques

- ✓ Syndrome pseudo-grippal
- ✓ choc cytokinique

Spécifiques

Parameter	Procedures
Screen (Tier I)	
Immunopathology	Hematology: complete blood count and differential
	Weights: body, spleen, thymus, kidney, liver
	Cellularity: spleen
	Histology: spleen, thymus, lymph node
Humoral-mediated immunity	Enumerate IgM antibody plaque-forming cells to T-dependent antigen (SRBC); LPS mitogen response
Cell-mediated immunity	Lymphocyte blastogenesis to mitogens (Con A) and mixed leukocyte response against allogeneic leukocytes
Nonspecific immunity	Natural killer (NK) cell activity
Comprehensive (Tier II)	
Immunopathology	Quantitation of splenic B- and T-cell numbers
Humoral-mediated immunity	Enumeration of IgG antibody response to SRBCs
Cell-mediated immunity	Cytotoxic T-lymphocyte (CTL) cytolysis; delayed hypersensitivity response (DHR)
Nonspecific immunity	Macrophage function [quantitation of resident peritoneal cells and phagocytic ability (basal and activated by MAF)]
Host resistance challenge Models (end points)[b]	Syngeneic tumor cells PYB6 sarcoma (tumor incidence)

Principaux modèles de résistance aux infections/tumeurs

Elément inoculé	Mécanismes immunitaires évalués
Listeria monocytogenes	Immunité à médiation cellulaire locale (macrophages, complément et lymphocytes T spécifiques) activité des neutrophiles et des NK en fin d'infection (Cohen, 2007 ; Keil *et al.*, 2001)
Streptococcus pneumoniae	Immunité innée (complément, macrophages, neutrophiles, cytokines des macrophages) (Keil *et al.*, 2001 ; Burleson et Burleson, 2007 ; Cohen, 2007)
Candida albicans	Immunité à médiation cellulaire et humorale (Germolec, 2004)
Plasmodium yoelli	Immunité à médiation cellulaire et humorale (Germolec, 2004)
Trichinellaspiralis	Immunité à médiation cellulaire et humorale (Germolec, 2004)
Cytomegalovirus	Immunité à médiation cellulaire (lymphocyte T cytotoxiques) et innée (activité des NK) (Garssen *et al.*, 1995), étude de la réactivation d'une maladie virale latente lors d'immunosuppression (Burleson et Burleson, 2007)
Influenza virus	Immunité à médiation cellulaire (lymphocyte T cytotoxiques), innée (activité des NK) (Garssen *et al.*, 1995)et humorale (Burleson et Burleson, 2007)
PYB6 sarcoma	Activité des cellules NK
B16F10	Activité des cellules NK

ARTICLE 2

In vitro studies on immunotoxic potential of Orange II in splenocytes

2.3. Splenocyte culture

Balb/c or Swiss mice were sacrificed according to the guidelines for the care and use of laboratory animals of Indian Institute of Toxicology Research, Lucknow, India. Spleens were taken out, washed with cold phosphate buffered saline and cell suspension was prepared by mincing tissue in incomplete Dulbecco's modified Eagles

2.4. Cytotoxicity assays

Cytotoxicity of Orange II in splenocytes was determined by MTT assay and by propidium iodide (PI) staining for 24, 48 and 72 h. MTT assay was done according to Mosmann (1983). For PI staining, cells were cultured and treated with different

2.5. Immunophenotyping

Cells were labeled with surface marker specific antibodies for the identification of individual populations of B cells and T cells in control and Orange II treated groups after 72 h of treatment. The cells were suspended in staining buffer (2% FBS, 1% sodium azide in PBS) and stained with Alexafluor 700-conjugated anti-CD19

2.6. Lymphoproliferation assays

Concanavalin A (Con A) and lipopolysaccharides (LPS) were used to induce blastogenesis and proliferation in T cell and B cell populations present in splenocytes culture, respectively. Cultured splenocytes were treated with Con A (5 μg/ml) or LPS (10 μg/ml) in presence of Orange II and incubated at 37 °C for 72 h. The relative fold

2.8. Cytokines analysis

Samples from Con A or LPS stimulated splenocytes were collected for the estimation of $T_H1/T_H2/T_H17$ cytokines by using Cytometric Bead Array Flow Cytometry Kit (BD biosciences, San Jose, CA). Samples were prepared for cytokines analysis as directed by the kit manufacturer and analyzed on the same day. CBA FCAP array software evaluated the level of cytokines present in the samples on the basis of standard curve obtained for each cytokine.

2.9. Data analysis

ARTICLE 3

Cytochrome P450 1B1 Is Required for 7,12-Dimethylbenz(a)-anthracene (DMBA) Induced Spleen Cell Immunotoxicity

Spleen cell preparation. Single cell suspensions were prepared from five individual mice per treatment group. Spleen cells were harvested as described previously (Burchiel *et al.*, 2004). In brief, spleens were isolated in RPMI

Lymphocyte mitogenesis assay. Lipopolyssaccharide (LPS) and concanavalin (Con A) were used to evaluate B and T cell proliferation, respectively. Spleen cells from individual mice were exposed to mitogens for three days in 96 well culture plates (200 µl @ 1×10^6 cells/ml) in replicates of six containing

In vitro plaque-forming cell assay. Mouse spleen cells collected sterilely (2×10^6 cell/ml, 0.5 ml) were cultured for four days with 0.5 ml of washed 1% sheep red blood cells (SRBC) (Colorado Serum, Denver, CO) in 48-well, flat-bottomed plates (Corning Glass, Corning, NY) with RPMI 1640 medium

Flow cytometric analysis. After spleen cells were harvested, 1×10^6 cells were aliquoted into three 12×75 mm tubes, and their surface marker expression was analyzed using a FACS Calibur Flow Cytometry system (Becton Dickinson Immunocytometry Systems, San Jose, CA). Three combinations of custom rat

Natural killer cell assay. To measure the nonspecific immunity of natural killing cells (NK), NK cell activity was quantitated by determining the ability of NK cells to lyse the NK sensitive Yac-1 target cells (ATCC, Manassas, VA). Briefly, the Yac-1 target cells, 2×10^6 cells/ml were suspended in complete RMPI 1640 medium and radiolabeled with sodium chromate (^{51}Cr) (Perkin-

ARTICLE 4

Advantages of Papio anubis for preclinical testing of immunotoxicity of candidate therapeutic antagonist antibodies targeting CD28

Flow cytometry

Fluorescent mAbs against mouse CD45 (30-F11) and human CD3 (SP34–2), CD4 (L200), CD8 (RPA-T8), CD25 (M-A251), CD28 (28.6), CD69 (FN50) and CD95 (DX2) were from BD

Endothelial cell co-culture assay

Human umbilical vein endothelial cell (HUVEC), used between the second and fifth passage, were originally obtained from Lonza and cultured in Endothelial Cell Basal Medium (Promocell) supplemented with 10% heat-inactivated human AB sera, 0.4% endothelial cell growth supplement/heparin (Promocell), hydrocortisone (1 µg/ml, Promocell), human basic fibroblast growth factor (1 ng/ml, Promocell), human epidermal growth factor (0.1 ng/ml, Promocell), 100 U/ml penicillin (Life Technologies) and 0.1 mg/ml streptomycin (Life

Anti-CD3 biological assay

10^5 human PBMC from healthy volunteers were cultured in 96-well U-bottomed microtiter plates (Nunc) precoated with 1 µg/ml of anti-CD3 antibody (OKT3) in complete medium (described above) supplemented with 2% heat-inactivated human AB sera, in the presence of 10 µg/ml of superagonist anti-CD28 mAb (ANC28.1, Calbiochem), divalent IgG anti-CD28 mAb (CD28.2, BD Biosciences), monovalent anti-CD28

Animals and treatments

Seven to ten week old IL-2rγ knockout mice (Charles River) were irradiated (2 Gy) and infused intraperitoneally (i.p.) with $50x10^6$ freshly isolated human or baboon PBMC from healthy donors, as previously described.[7] Mice were then maintained in aseptic conditions and were monitored every week for T-lymphocytes engraftment in the blood. Two weeks after PBMC injection, mice were treated once i.p. with 50 µg of superagonist anti-CD28 (ANC28.1), 150 µg of anti-CD28 Fab' fragment (FR104), or equivalent volume of excipient. Retro-orbital blood

ARTICLE 5

Cetuximab-Induced Anaphylaxis and IgE Specific for Galactose-α-1,3-Galactose

METHODS

We analyzed serum samples from four groups of subjects for IgE antibodies against cetuximab: pretreatment samples from 76 case subjects who had been treated with cetuximab at multiple centers, predominantly in Tennessee, Arkansas, and North Carolina; samples from 72 control subjects in Tennessee; samples from 49 control subjects with cancer in northern California; and samples from 341 female control subjects in Boston.

CASE DEFINITION AND GRADING SYSTEM

Our case definition and grading of hypersensitivity reactions were based on documented symptoms listed in the National Cancer Institute Common Toxicity Criteria, version 3.[11,16] The characteristics of a grade 1 reaction were transient flushing or rash with a fever of less than 38°C (100.4°F); those of

IMMUNOCAP IgE ASSAYS

ImmunoCAP is a variation of the radioallergosorbent test in which IgE antibodies that have bound to antigen on the solid phase are detected with a secondary enzyme-labeled anti-IgE antibody.[14,20] Total and specific IgE antibodies were measured

EVALUATION OF ANTIGENS

Cetuximab, which is produced by expressing clone C225 in the mouse myeloma cell line SP2/0, was provided by ImClone Systems.[8,17] A variant of cetuximab, CHO-C225, which is produced in Chinese hamster ovary (CHO) cell lines, was also obtained from ImClone. CHO cells do not produce α-1,3-galactosyltransferase and, for this reason, have a pattern of glycosylation that differs from that of cetuximab.[17,18] This monoclonal antibody, which

ARTICLE 6

Innate immunity drives xenobiotic-induced murine autoimmune cholangitis

Experimental mice

Female C57BL/6 mice aged 8–10 weeks were obtained from the National Laboratory Animal Center, Taipei, Taiwan. B6.129S6-Cd4^{tm1knw}/J (CD4$^{-/-}$) and B6.129S2-*Cd8a*^{tm1Mak}/J (CD8$^{-/-}$) mice were purchased from The Jackson Laboratory

Determination of anti-PDC-E2 antibodies

Serological IgM and IgG anti-PDC-E2 were measured by ELISA using recombinant mouse PDC-E2. Briefly, purified recombinant mouse PDC-E2 at 1 μg/ml in carbonate buffer (pH 9·6) was coated onto ELISA plates at 4°C overnight. After blocking with 1% casein (Sigma-Aldrich) for 1 h,

Mononuclear cell preparation

Livers were perfused with PBS containing 0·2% BSA (PBS/0·2% BSA) (Sigma-Aldrich), passed through a 100 μm nylon mesh, and resuspended in PBS/0·2% BSA. The paren-

Flow cytometry

Subsets of liver mononuclear cells were measured by flow cytometry. In all cases, we used a previously optimally defined dilution of monoclonal antibodies. Before staining, all cells were preincubated with anti-CD16/32 (clone 93) to

Histopathology

Portions of the liver were excised and fixed immediately with 10% buffered formalin solution for 2 days at room temperature. Paraffin-embedded tissue sections were then cut into 4-μm slices for routine haematoxylin and eosin (H&E) and Masson's trichrome staining. Liver inflammation was evaluated under a microscope.

LES DIFFÉRENTES FORMES D'IMMUNOTOXICITÉ

- ✓ **Immunosuppression**
- ✓ **Immunostimulation**

← Immunotoxicité directe

- ✓ **Hypersensibilité**
- ✓ **Auto-immunité**

← Immunotoxicité indirecte

I-IMMUNOSUPRESSION

Altération du système immunitaire conduisant à une diminution/supression de la réponse immune.

Cibles, effets et conséquences (TD)

Cibles

Effecteurs du SI

Effets

Mort cellulaire ou perte de fonctionnalité

Conséquences

Fonction de l'effecteur dans la RI

Effets néfastes de l'exposition aux AI:

- *Infections virales, bactériennes, parasitaires*
- *Lymphomes , Leucémies*

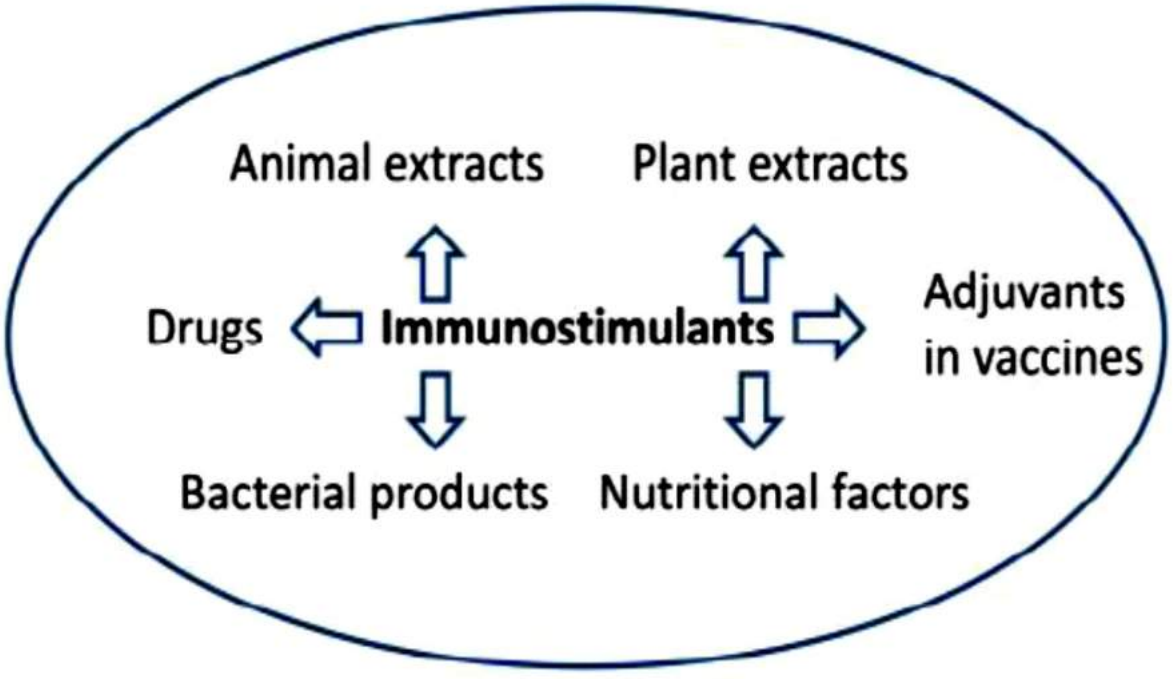
Developmental Immune Event	Xenobiotics	Immunotoxic Effect(s)	Increased Disease Risk	References
Macrophage colony formation activity; seeding of tissues, and homeoregulatory function	Lead; Chlordane	Hyper-inflammatory testicular macrophages and defective myeloid cell repopulation; Decreased colony forming units	Male sterility; Infectious diseases	Pace et al.,2005; Blyler et al., 1994; Barnett et al., 1990
Seeding of the thymus and expansion of thymocyte populations	PAHs; Cyclophospha-mide; DES; Nicotine	Thymocyte depletion; thymic atrophy; Increased Apoptosis	Cancer	Holladay and Smith, 1994; Prakash et al., 2007; Besteman et al., 2005; Middlebrook et al., 2002; Rodriguez et al. 1999;
Clonal selection in the thymus and induction of apoptosis	TCDD	Increased apoptosis; Thymic atrophy	Infectious diseases; autoimmunity	Camacho et al., 2004; 2005; Fisher et al., 2005; Vordrestrasse et al., 2006; Holladay, 1999
Altered pattern of Treg (CD4+ CD25+ high) generation, seeding and activation	Bisphenol A; Cyclophospha-mide	Reduced number of Tregs	Loss of tolerance; Autoimmunity	Ohshima et al., 2007; Lutsiak et al., 2005
Dendritic cell maturation to promote Th1	Lead; DEX; Maternal smoking;	Promotion of Th2 responses with reduced Th1 promotion	Allergic disease/ Asthma	Gao et al., 2007; Mainali and Tew; 2004; Pachlopnik et al., 2007; Hamada et al., 2007
Th1 functional increases upon birth	Cigarette smoke; Sucralfate	Reduced CTL activity; reduced Th1 cytokines; increased IgE; Th2 bias in cytokines	Cancer, Allergic disease; Airway hyper-responsiveness; Childhood respiratory infections	Ng et al., 2006; Wang et al., 2007; Penn et al., 2007; Haburg et al., 2007; Scholl et al., 2007
Surfactant-induced macrophage alterations; toll-like receptor maturation	Ethanol	Defective surfactant production; alveolar macrophage function; toll-like receptor maturation	Childhood respiratory diseases; neonatal infections	Ping et al., 2007; Lazic et al., 2007; Gauthier et al., 2005; Sadeghi et al., 2007

*Modified from Dietert and Piepenbrink (2006). PAHs = polycyclic aromatic hydrocarbons, TCDD = 2,3,7,8, tetrachlorodibenzo-p-dioxin, DES = diethylstilbesterol, DEX = dexamamethasone.

II-IMMUNOSTIMULATION

Stimulation du système immunitaire conduisant à une **augmentation** ou **un dérèglement** de la réponse immune.

Les agents immunostimulants



Exemple : Les agents biologiques

A c monoclonaux, cytokines, antagonistes de récepteurs

- AC anti-CD20 (rituximab, Mabthera°)
- AC anti-intégrine a 4 (natalizumab, Tysabr°)
- AC LFA-1 (Efalizumab, Raptiva°)
- AC anti-IL6 (tocilizumab, Actemra°)
- AC anti-IL12 (ustekinumab, Stelara°)

- TNF
  - anticorps (infliximab, adalimumab)
  - Récepteur soluble (etanercept)
- Autres cytokines
  - antagoniste du récepteur de l'IL1 (anakinra)
  - inhibiteur de l'ICE
  - anticorps anti-récepteur de l'IL6 (MRA)
- Lymphocytes T
  - CTL A4-Ig (abatacept)

Agents biologiques

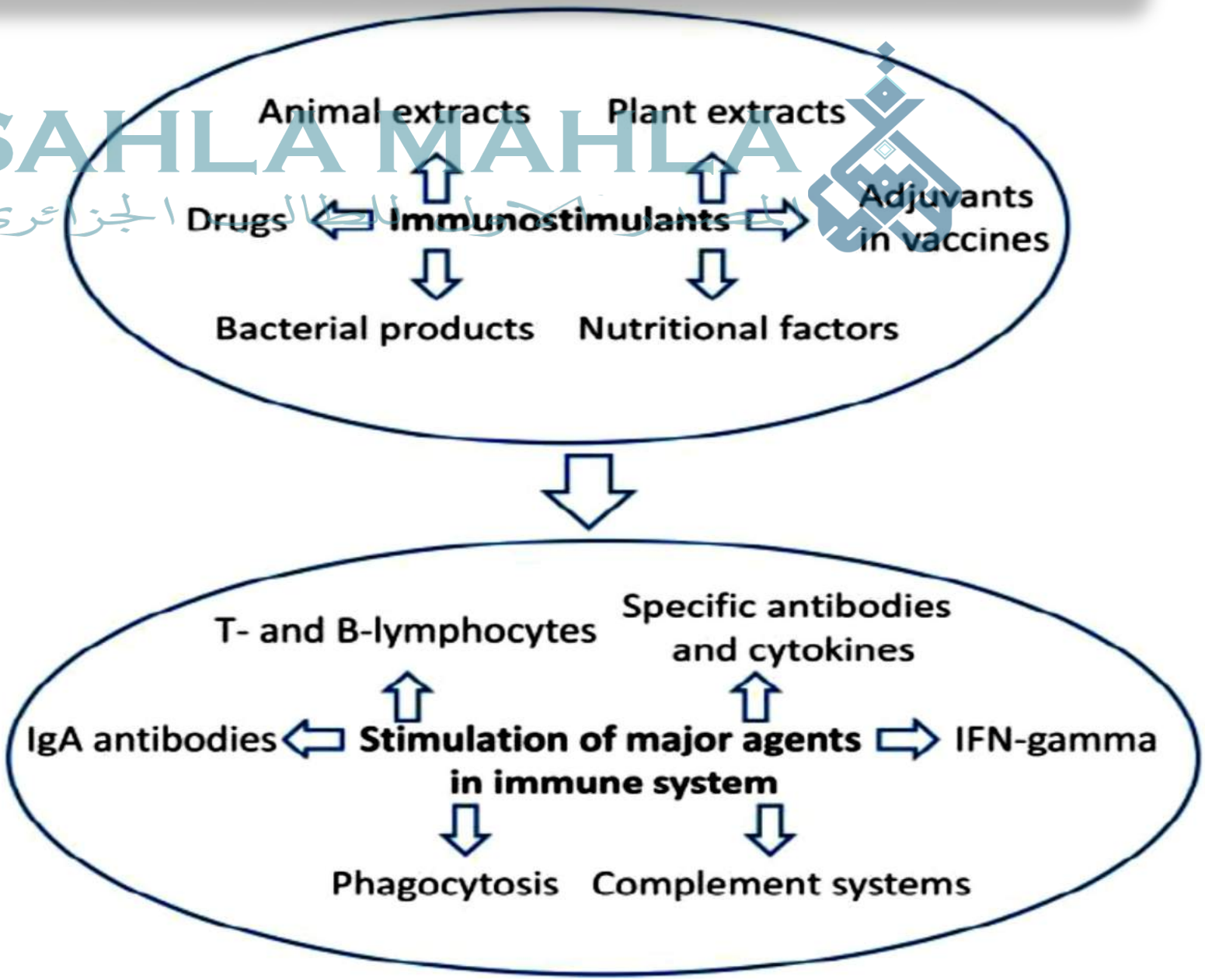
Trade name	Generic (INN) name	Species/ isotype	Target	Indication(s)	RoA	Regime*	First approved (Year)
#Orthoclone-OKT3®	Muromonab CD3	Mouse IgG2a	CD3	Organ rejection (renal, heart, liver)	IV	5 mg daily	FDA (1986) EU (1987)
ReoPro®	Abciximab	Chimeric Fab	gpII/IIIa	PCI	IV	0.25 mg/kg before PCI then 0.125 µg/kg/mi for 12 h.	FDA (1994) EU (1994)
#Zenapax®	Daclizumab	Humanized IgG1	IL-2R	Organ rejection (renal)	IV	1 mg/kg before surgery then every 2 weeks for a total of 5 doses	FDA (1997) EMA (1999)
Rituxan®, Mabthera®	Rituximab	Chimeric IgG1	CD20	Cancer (CD20+ NHL), RA	IV	NHL: 375 mg/m²/wk for 4–8 wks RA: 2 x 1,000 mg, 2 weeks apart, then every 24 wks	FDA (1997) EMA (1998)
Simulect®	Basiliximab	Chimeric IgG1	IL-2R	Organ rejection (renal)	IV	20 mg before and after surgery	FDA (1998) EMA (1999)
Synagis®	Palivizumab	Humanized IgG1	RSV	RSV infection	IM	15 mg/kg/mo during RSV season (≤6 mo)	FDA (1998) EMA (1999)
Herceptin®	Trastuzumab	Humanized IgG1	ErbB2 (HER-2)	Cancer (HER2+ breast)	IV	2 mg/kg/wk for 12 wks then 6 mg/kg every 3 wks	FDA (1998) EMA (2000)

Mylotarg™	Gemtuzumab ozogamicin	Humanized IgG4-calichemicin	CD33	Cancer (AML)	IM	9 mg/m^2, 2 doses 2 weeks apart	FDA (2000)
Campath®	Alemtuzumab	Humanized IgG1	CD52	Cancer (CLL), RA, MS	IV	30 mg, 3 times/wk for 12 wks	FDA (2001) EMA (2001)
Humira™	Adalimumab	Human IgG1	TNFα	RA, CrD, AS, Ps, PsA, JIA	SC	40 mg/2 wks	FDA (2002) EMA (2003)
Zevalin®	Rituximab + Ibritumomab tiux-etan (IT) (In^{111}/Y^{90})	Mouse IgG1	CD20	Cancer (NHL)	IV	Rituximab 250 mg/m^2 then 5 mCi IT In^{111} (day 1) or 0.4 mCi IT Y^{90} (day 7)	FDA (2002) EMA (2004)
Xolair®	Omalizumab	Humanized IgG1	IgE	Allergic Asthma	SC	150–375 mg/2–4 wks	FDA (2003) EMA (2005)
Bexxar®	Tositumomab (I^{131})	Mouse IgG2a	CD20	Cancer (NHL)	IV	450 mg Tositumomab then I^{131} Tositumomab (35 mg) delivering 75 cGy total body irradiation	FDA (2003)
#Raptiva®	Efalizumab	Humanized IgG1	CD11a (LFA-1)	Ps	SC	0.7 mg/kg, then 1 mg/kg/wk	FDA (2003) EMA (2004)
Amevive®	Alefacept	Human LFA-3-FcIgG1	CD2	Ps	IM	15 mg/wk for 12 wks	FDA (2003)
Erbitux™	Cetuximab	Chimeric IgG1	EGFR	Cancer (CRC, SCCHN)	IV	250–400 mg/m^2/wk for 6–7 wks	FDA (2004) EMA (2004)

Comparaison des médicaments traditionnels et les agents biologiques

Traditional drugs	Biological agents
Small molecule (MW < 1 kDa)	Large complex molecules (MW >> 1 kDa)
Synthetized chemicals (xenobiotics)	Structurally similar to autologous proteins Produced with molecular genetic technique and purified from engineered cells
Stable	Heat sensitive
Well characterized and homogeneous	Heterogeneous composition
Metabolized to active and inactive products	Digested and processed, not metabolized Catabolized to endogenous amino acids
Cytochrome P450 involvement	Cytochrom P450 independent
Multiple drug interactions	No drug interactions
Oral administration possible	Oral administration not possible
Linear dose–response	Non linear dose–response
Pharmacological effect	Biological effect

Fonctions des immunostimulants



Conséquences néfastes de l'immunostimulation

-Une réaction hyperthermique pseudo-grippale

- Choc cytokinique: conduit à un déséquilibre immunitaire et une défaillance multiviscérale (TGN1412).

- L'apparition des réactions d'hypersensibilités et/ou des maladies auto-immunes

Classification des effets néfastes des agents biologiques (Thérapeutiques)	
Type α	Réactions liées au syndrome cytokinique
Type β	Réactions d'hypersensibilités immédiate et retardée
Type γ	Réactions liées au syndrome d'imbalance immunitaires
Type δ	Réactions liées à l'expression du même Ag sur différents tissus
Type ε	Réactions non-immunologiques

Classification des effets néfastes induits par les anti-TNF, traitement commun dans le traitement des maladies inflammatoires

Agents biologiques	Effets	Classification
Anti-TNF Infliximab Adalimunab certolizumab	Hypersensibilité retardée	Type β
	Auto-immunité (lupus)	Type γ
	Défaillance cardiaque	Type ε

Classification des effets néfastes induits par les agents biologiques communs dans le traitement des maladies inflammatoires

Biological agent	Adverse effect	Classification	
Anti-TNF	Acute HSR (local and systemic)	Type beta reaction	β
Infliximab	Delayed HSR (serum sickness		
Adalimumab	disease)	Type gamma reaction	γ
Certolizumab	Infection	Type gamma reaction	γ
	Paradoxical adverse effects: vasculitis, colitis, psoriasis-like eruption, etc....	Type gamma reaction	γ
	Autoimmunity: lupus, hepatitis, thyroiditis, etc..	Type gamma reaction	γ
	Heart failure	Type epsilon reaction	ε
Anti- alpha-4 integrin	Acute HSR (local and systemic)	Type beta reaction	β
Natalizumab	Delayed HSR (serum sickness disease)	Type gamma reaction	γ
	Infection: progressive multifocal leukoencephalopathy	Type gamma reaction	γ
	Autoimmunity: hepatitis, thyroiditis		
Anti-IL12 / anti-IL23	Delayed HSR (serum sickness disease)	Type beta reaction	β
Ustekinumab	Infection	Type gamma reaction	γ

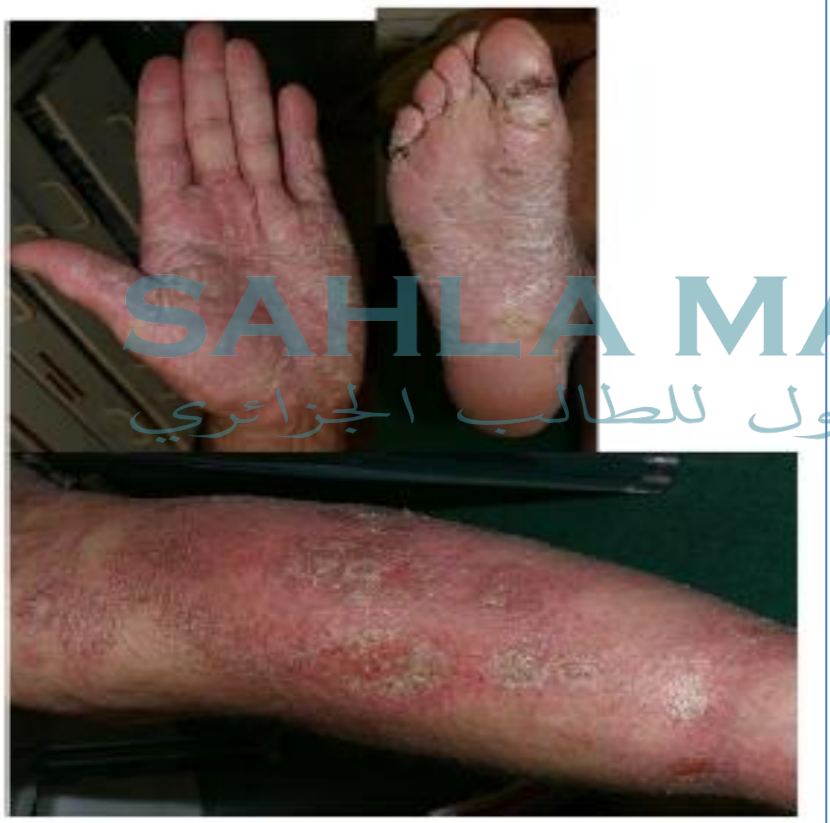


Figure 4 Paradoxical psoriasiform eruption (type γ) durin adalimumab treatment in rheumatoid arthritis.

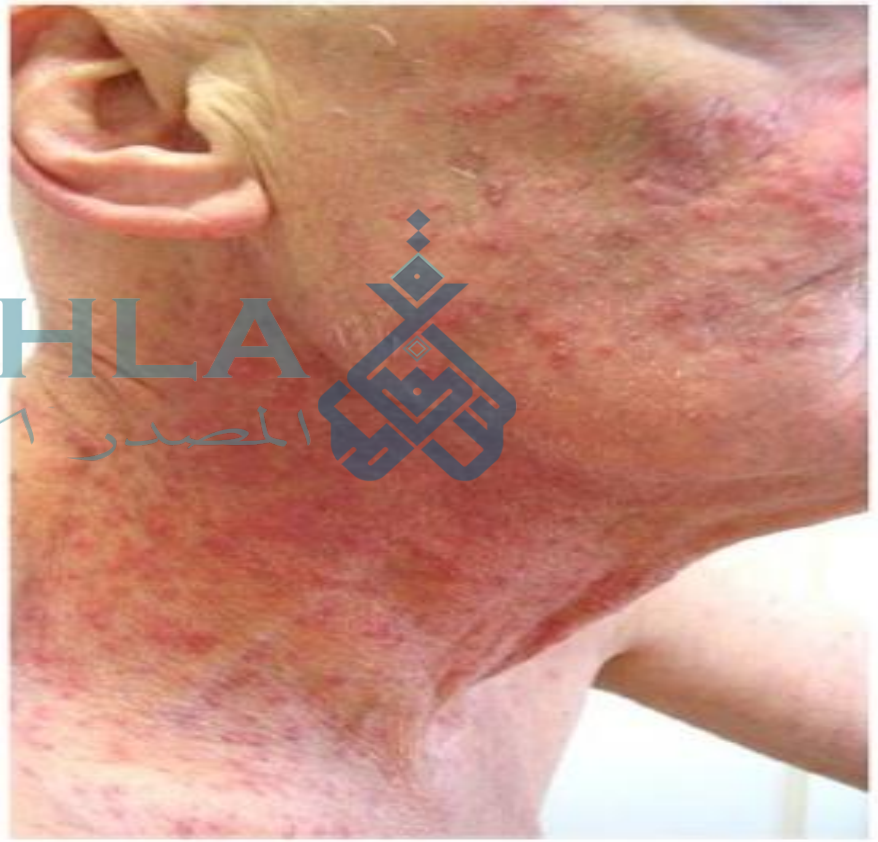
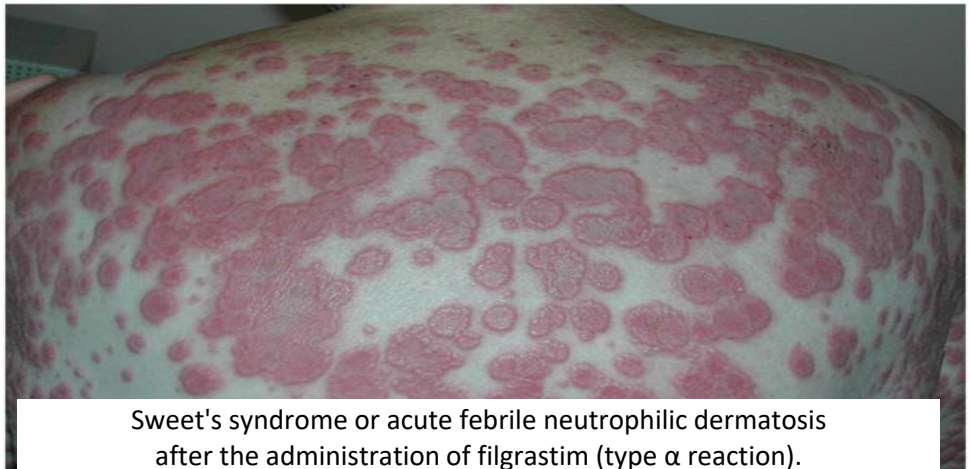


Figure 5 Acneiform eruption (type δ reaction) during cetuximab treatment in head and neck.



Sweet's syndrome or acute febrile neutrophilic dermatosis after the administration of filgrastim (type α reaction).

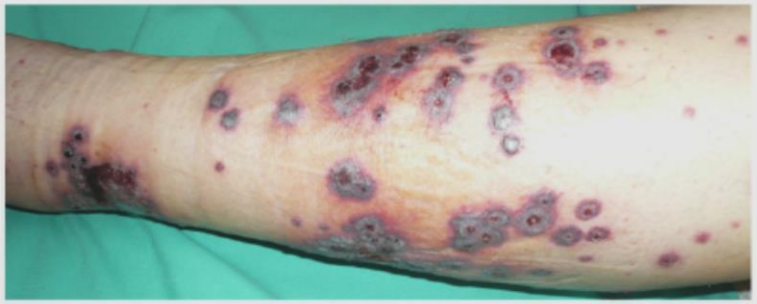


Figure 3 Cutaneous vasculitis (type β) during adalimumab treatment in Crohn's disease.

III-LES HYPERSENSIBILITÉS (HS)

Récapitulatif des différents types de réactions allergiques

Type		Délai	Mécanisme	Clinique
Type I	Hypersensibilité médiée par les IgE	2-30 min	Pontage des IgE par l'allergène entraînant une activation des mastocytes	• Anaphylaxie systémique • Réaction clinique immédiate locale
Type II	Hypersensibilité médiée par les IgG	5-8 h	Ac cytotoxique (C, ADCC) ou Ac anti-récepteurs cellulaires	• Réactions transfusionnelles • Anémies hémolytiques auto-immunes
Type III	Hypersensibilité due aux IC	2-8 h	Dépôt d'immuns complexes	• Maladie sérique…
Type IV	Hypersensibilité retardée	24-72 h	Immunité cellulaire liée à des LT CD4 (Th1, Th2) et des LT CD8 (cytotoxiques)	• Dermatite de contact • Rejet de greffe

Produits impliqués dans les réactions d'hypersensibilités

Classification	Molécules
Hypersensibilité immédiate (HSI)	Béryllium (alliages), Isocyanates Anhydride triméllitique, β-lactames, sulfamides
Hypersensibilité cytolytique (Type II)	Anhydride triméllitique, mercure, quinine, quinidine nitrofurantoine pénicilline
Hypersensibilité à complexes immuns (Type III)	Anhydride triméllitique, mercure pénicillines, sulfamides, streptomycine
Hypersensibilité retardée à médiation cellulaire (Type IV)	DNCB, Béryllium, Chrome, Nickel pénicillines, sulfamides
Autres	Sulfamides, anticonvulsiants, AINS

Immunotoxicité par HS

Environnement

Expositions à des allergènes

Sensibilité génétique

Sensibilisation allergique

Réactions d'HS

-Immunotoxicité par mécanisme pseudo-allergique

-Immunotoxicité par mécanisme allergique (HSI)

Les Xé et des métabolites intermédiaires (issus des processus de biotransformation des Xé) jouent le rôle d'haptènes seraient à l'origine des réactions d'HS.

Formation d'Ac dirigés contre l'épitope Xé/metabolite

HSI: Mémorisation des Ac Lors d'un 2ème contact avec le xénobiotique il y a libération massive des médiateurs inflammatoires

IV-L'AUTO-IMMUNITE (AI)

Facteurs Environnementaux

Facteurs génétiques

Origine multifactorielle

Rupture

Auto-immunisation

Activation des lymp auto-réactifs

Tolérance → Auto-immunité

Maladies spécifiques d'organe

Anémies hémolytiques auto-immunes (Ac anti-Rhésus)

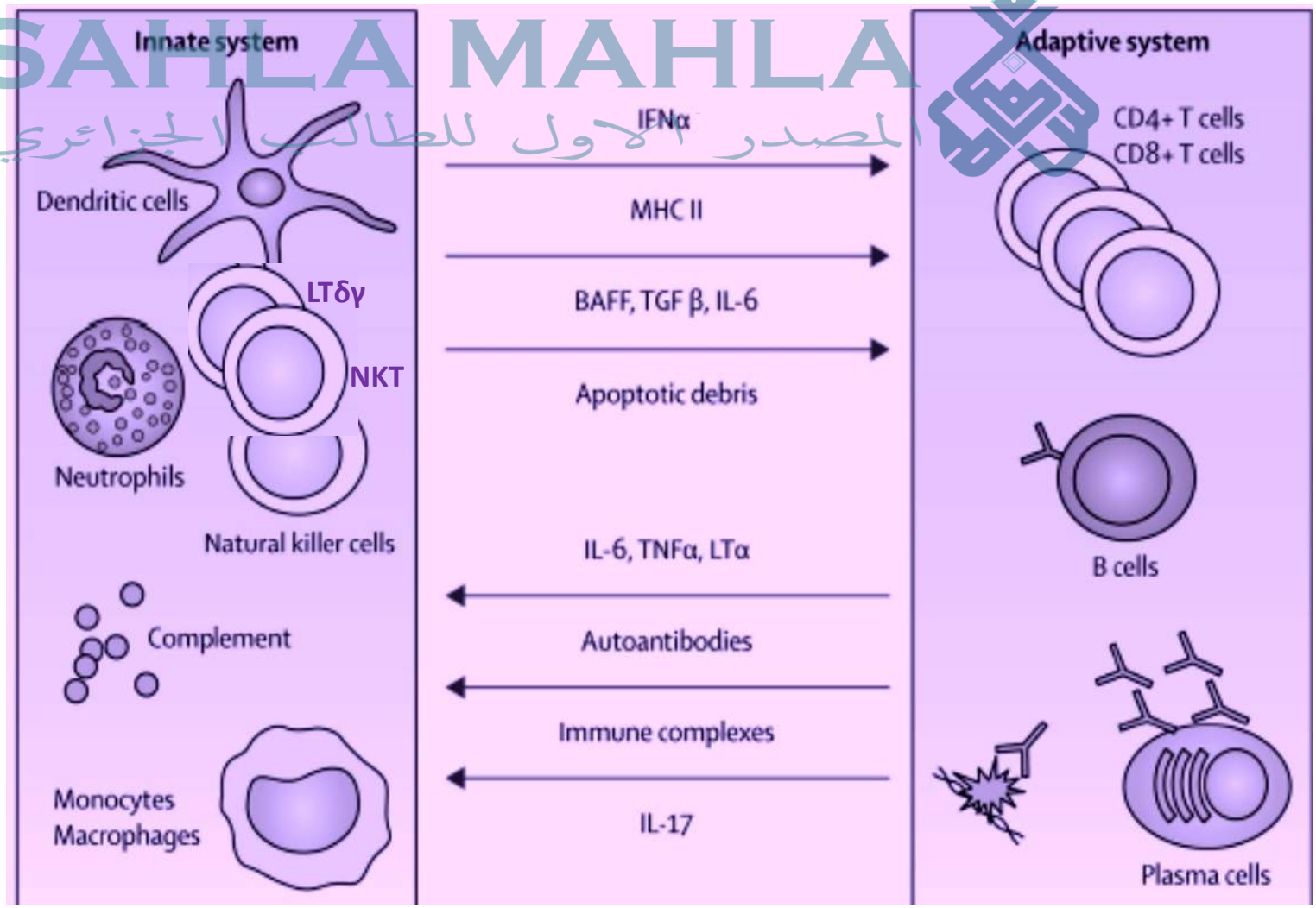
Maladies systémiques

Lupus érythémateux disséminé *(Ac anti-nucléaires)*

Exemples de maladies auto-immunes secondaires à l'expositions à certains xénobiotiques

Halothane (anesthésique métabolisé par le foie formant le trifluoroacétylé capable de se lier à des Ag de soi	→	HÉPATITE AUTO-IMMUNE
Hydralazine, sels d'or, formaldéhyde, interférons et silicone	→	LUPUS
Acide 2- octynoïque (2-OA (article 6)	→	CHOLANGITE BILLIAIRE AUTOIMMUNE

Interactions entre immunité innée et adaptative dans les MAI



Mimétisme moléculaire

Similitudes de structure entre un Ag exogène et un Ag du soi

Quelques exemples de mimétisme moléculaire

Maladies	Antigènes infectieux	Auto-antigènes
Rhumatisme articulaire aigu	Protéine M du streptocoque β hémolytique	Myosine
Maladie de Chagas	*Trypanosoma cruzi*	Cœur
Syndrome de Guillain et Barré	*Campylobacter jejuni*	Gangliosides de la myéline des nerfs périphériques
Diabète de type 1	Virus Coxsackie B4	GAD (*Glutamic Acid Decarboxylase*)

Médicaments et MAI

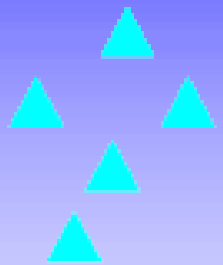
Biologics and drug-induced autoimmunity:

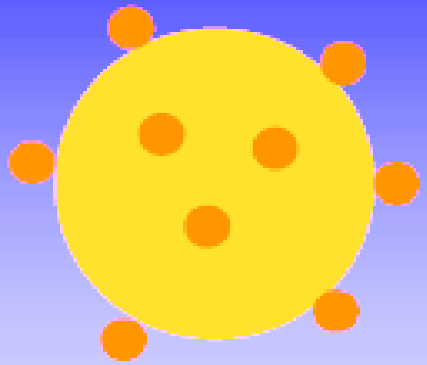
Drug	Classification	Indications	Newly induced autoantibodies	Clinical features/diseases	Possible mechanism of action
Infliximab (Remicade)	Anti-TNFα monoclonal antibody	Psoriasis, Crohn's disease, AS, PA, RA, UC	Anti-dsDNA, neucleosomes, ANA, Anti-cardiolipin	Fever, skin rash, arthalgias, malaise/Leukocytoclastic vasculitis, DIL	Apoptosis, alteration of cytokine profile
Etanercept (Enbrel)	TNFα soluble receptor-fusion protein	RA, JIA, PA, AS, moderate to severe plaque psoriasis	Anti-dsDNA, neucleosomes, ANA, Anti-cardiolipin	Fever, skin rash, arthalgias, malaise/Leukocytoclastic vasculitis, DIL	Apoptosis, alteration of cytokine profile
Adulimumab (Humira)	Anti-TNFα monoclonal antibody	RA, PA, AS, Crohn's disease, moderate to severe chronic psoriasis, JIA	Anti-dsDNA, neucleosomes, ANA, Anti-cardiolipin	Fever, skin rash, arthalgias, malaise/Leukocytoclastic vasculitis, DIL	Apoptosis, alteration of cytokine profile
Certolizumab (Cimzia)	PEGylated Fab' fragment of a humanized monoclonal antibody	Crohn's disease	Not known	Not known	Apoptosis, alteration of cytokine profile
Interferon-α-2b	Pegolated interferon	Hepatitis B & C, melanoma, other malignancies	Antithyroid antibodies, microsomal thyroid antigen, thyroglobulin, ANA, anti-smooth muscle	Autoimmune thyroid disease	Affecting Th1/Th2 balance, modulation of cytokine profile
IL2	Recombinant IL-2	Metastatic cancer	RF, ANA	Inflammatory arthritis	Enhancement of expression of HLA class II antigen

Mécanisme général

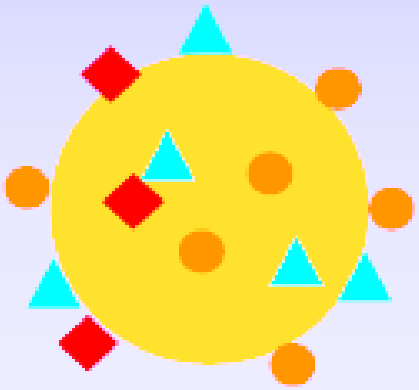
1- Formation de l'antigène

Xénobiotique
= haptène



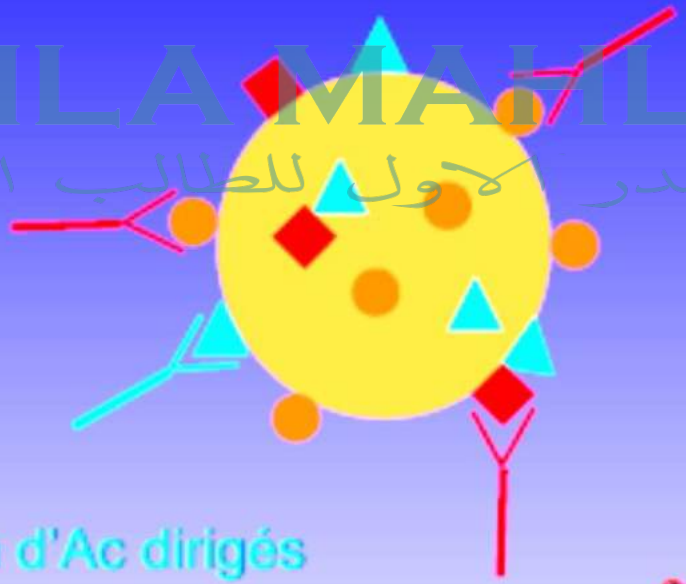


Constituant
du soi



Constituant du soi
modifié

2- Reconnaissance par le SI



3 épitopes:

Constituant du soi

Constituant du soi modifié

Xénobiotique

Formation d'Ac dirigés contre l'épitope xénobiotique

Mémorisation de ces Ac

Lors d'un deuxième contact avec le xénobiotique il y a libération massive d'anticorps

= HYPERSENSIBILITE

Formation d'Ac dirigés contre l'épitope constituant du soi modifié

Ac peu spécifique reconnaît aussi les constituants du soi non modifiés

= AUTOIMMUNITE

Mécanismes impliqués dans les altérations des RI par les Xé