Low Dose Medicine: a new pharmacological paradigm for the immunomodulation

Roundtables around the world

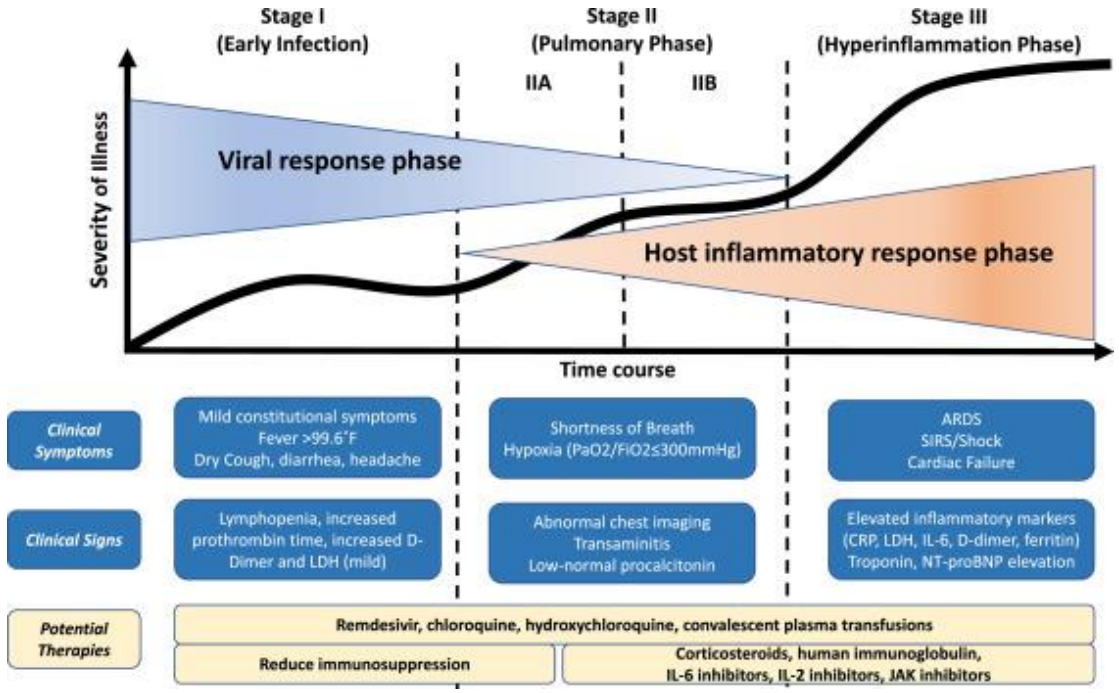
May 22th 2020

Our (unique) goal

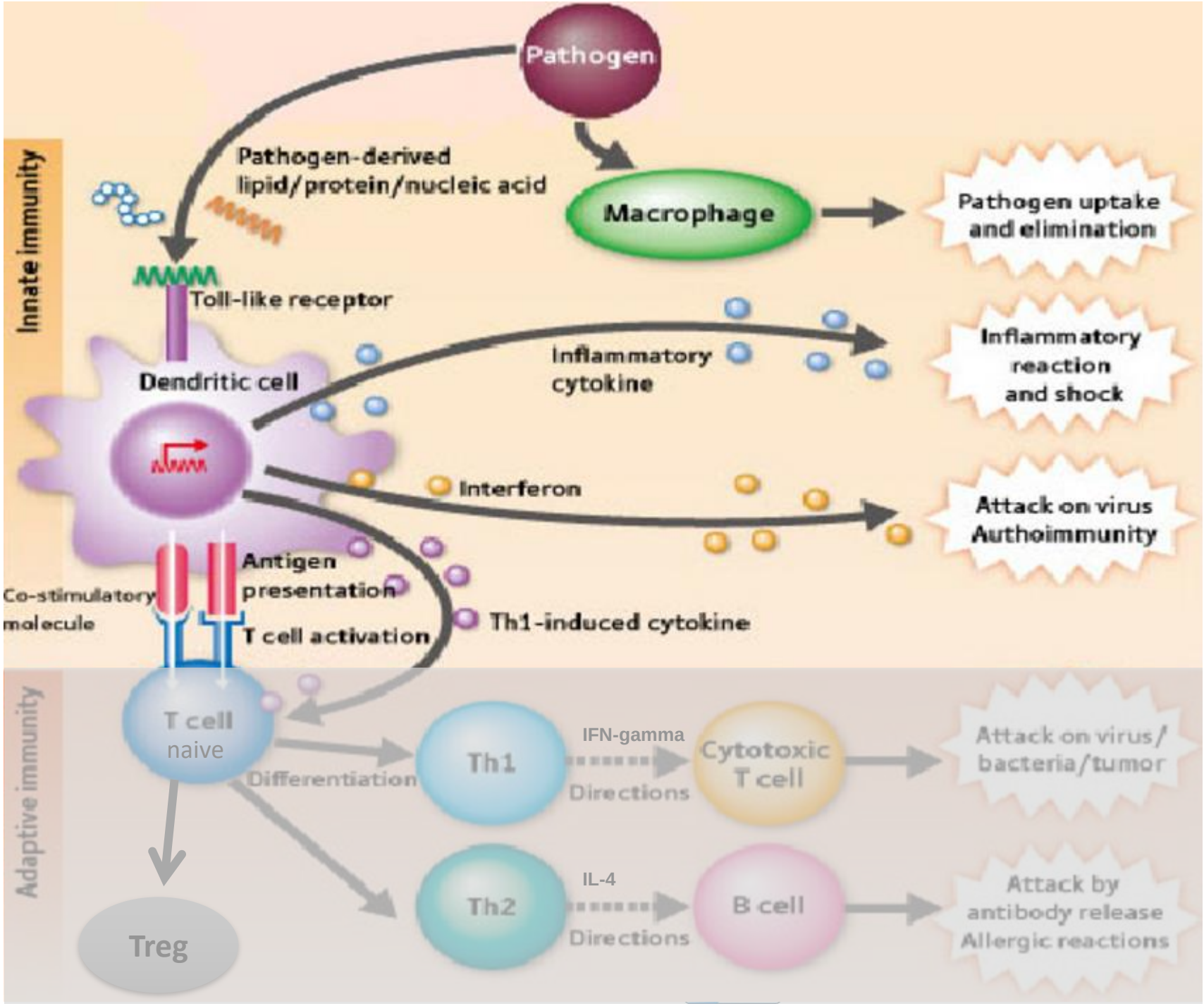
- *To immunostimulate without inflamming*
- *To reduce the inflammation without immunosuppressing*

Our (unique) goal

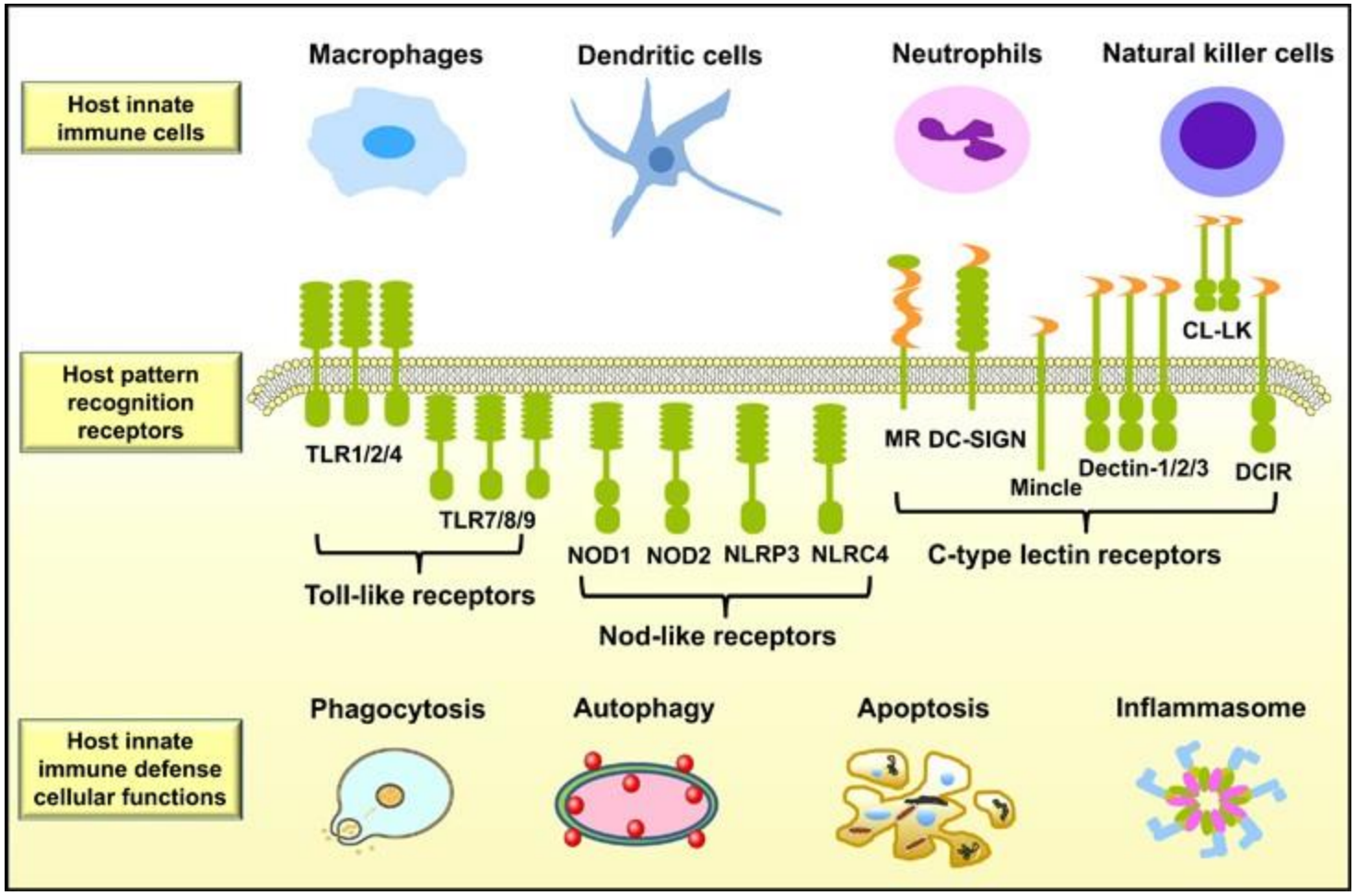
- *Before*
- *During*
- *After*



The bag of tools

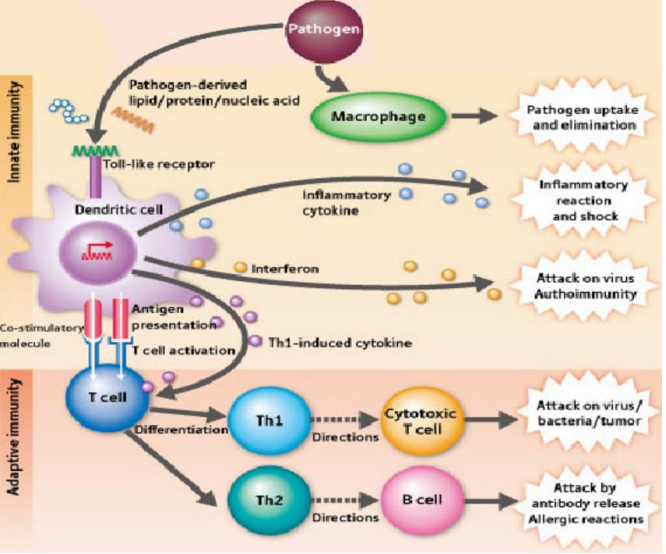

Pathogen
Pathogen-derived
lipid/protein/nucleic acid
Innate immunity
Macrophage
Pathogen uptake
and elimination
Toll-like receptor
Dendritic cell
Inflammatory
cytokine
Inflammatory
reaction
and shock
Interferon
Attack on virus
Authoimmunity
Antigen
presentation
Co-stimulatory
molecule
T cell activation
Th1-induced cytokine
Adaptive immunity
T cell
naive
Differentiation
Th1
IFN-gamma
Directions
Cytotoxic
T cell
Attack on virus/
bacteria/tumor
Th2
IL-4
Directions
B cell
Attack by
antibody release
Allergic reactions
Treg

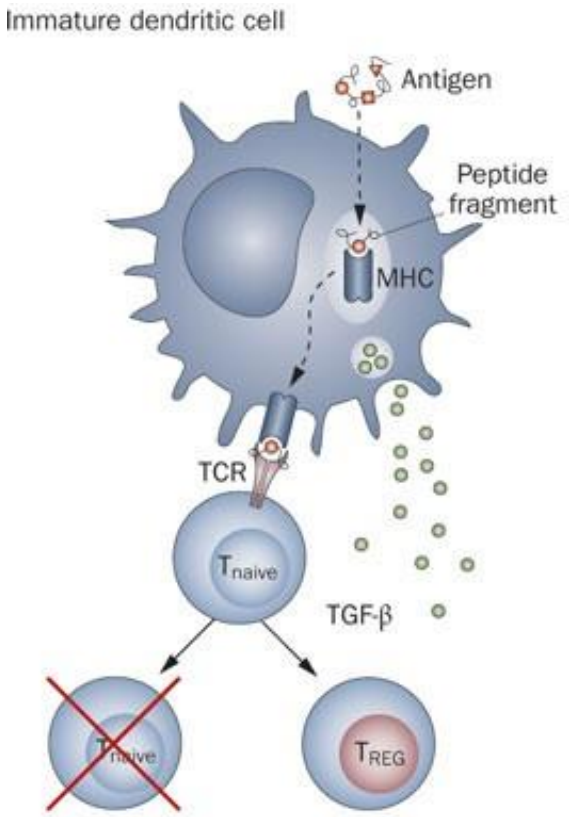
INNATE IMMUNITY

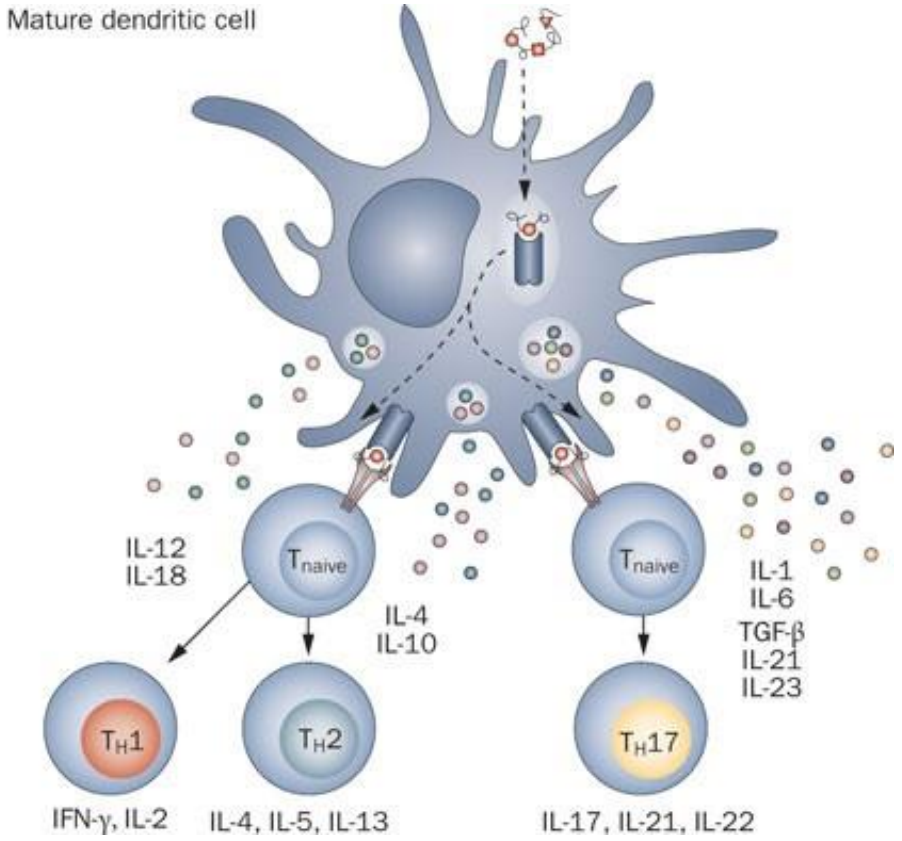


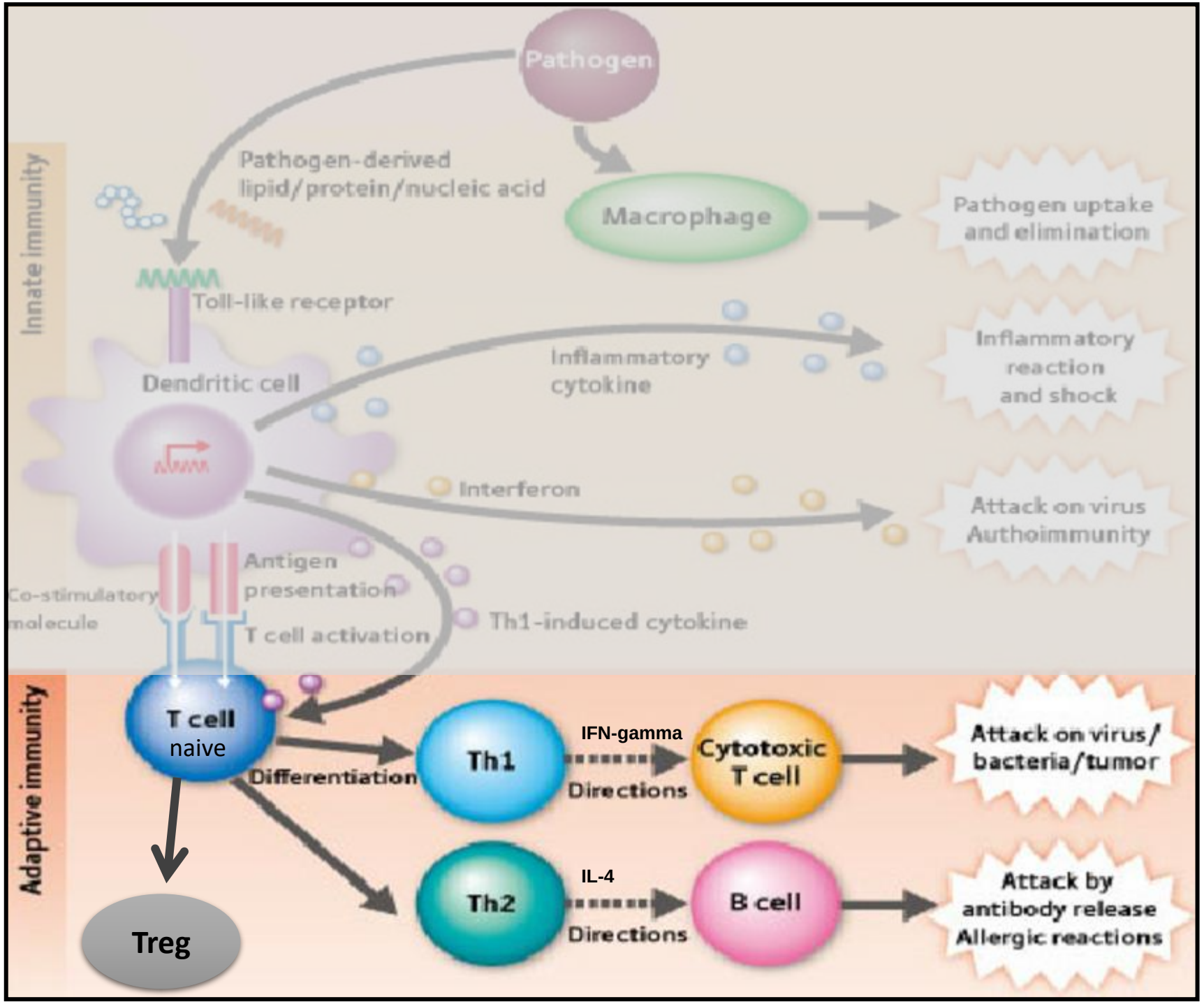
DENDRITIC CELLS

The link between innate ad adaptive immunity





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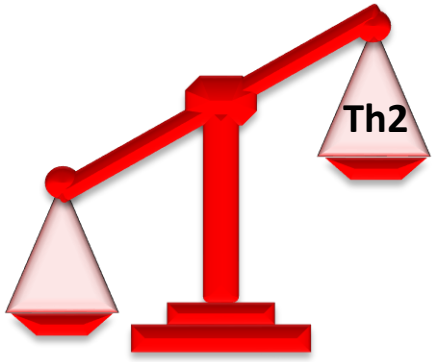
Antigen
presentation
Co-stimulatory
molecule
T cell activation
Th1-induced cytokine
Adaptive immunity
T cell
naive
Differentiation
Th1
IFN-gamma
Directions
Cytotoxic
T cell
Attack on virus/
bacteria/tumor
Th2
IL-4
Directions
B cell
Attack by
antibody release
Allergic reactions
Treg

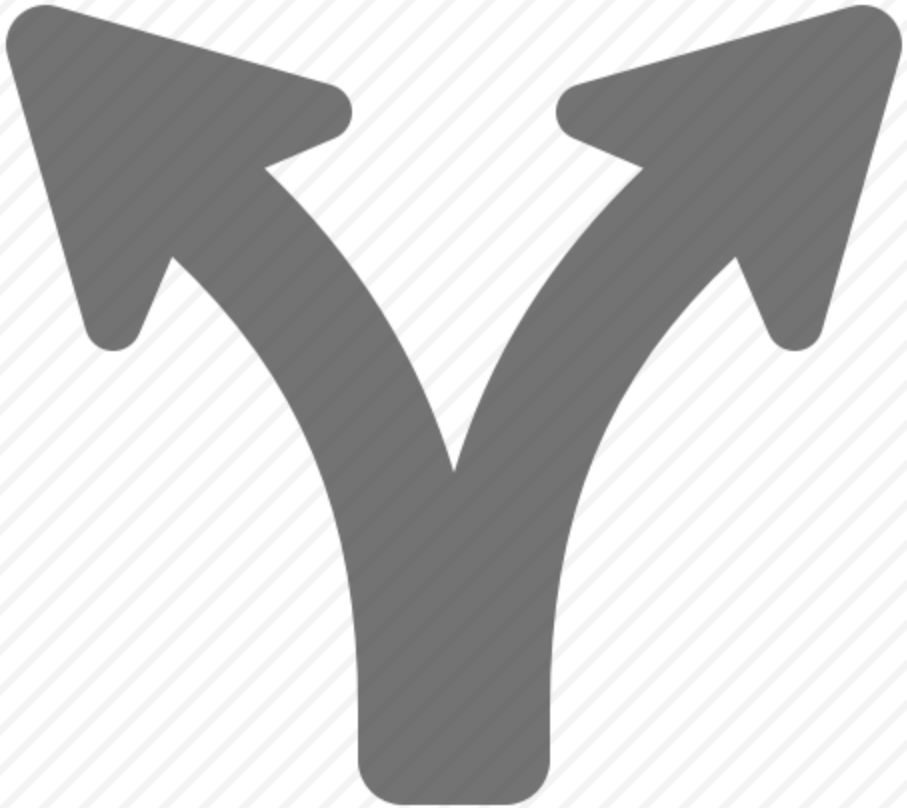
ADAPTIVE IMMUNITY

HUMORAL ANTIBODY DRIVEN IMMUNE RESPONSE

- **Th2 DRIVEN**

  IL-4
- B CELLS
- ANTIBODIES



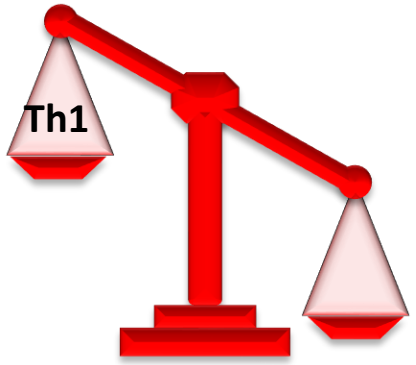


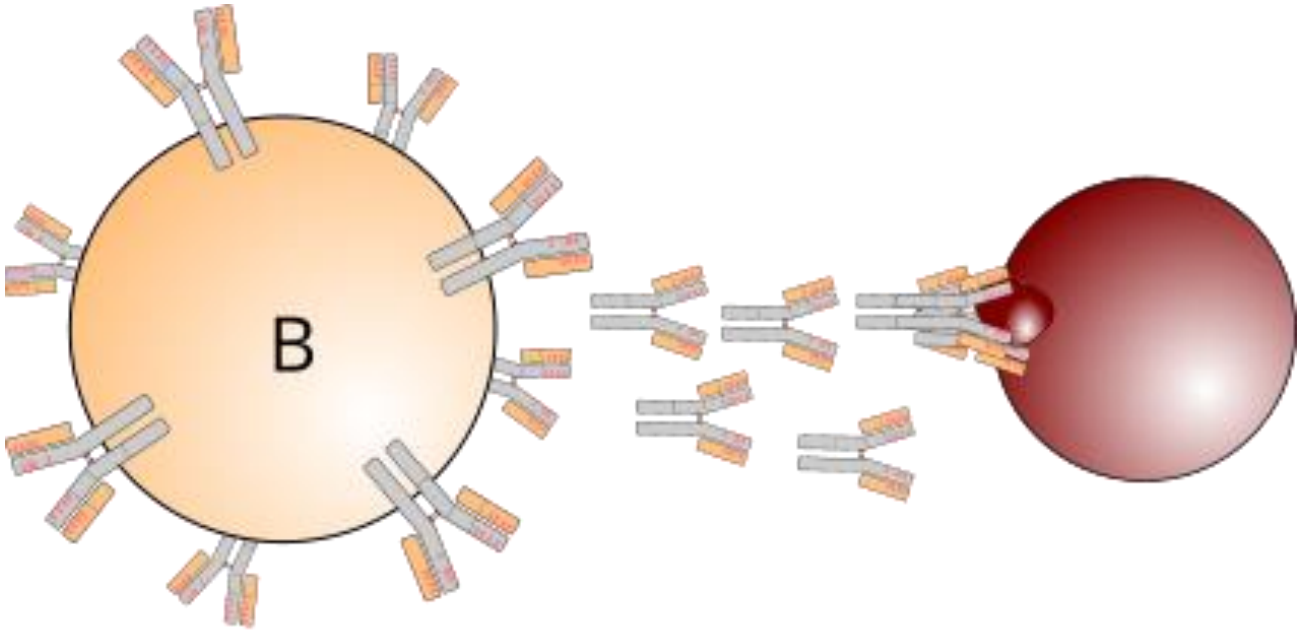
IMMUNE RESPONSE

CELL MEDIATED IMMUNE RESPONSE

- **Th1 DRIVEN**

  IFN-ϒ
- T CELLS – NK CELLS
- CITOTOXICITY





The viral infection

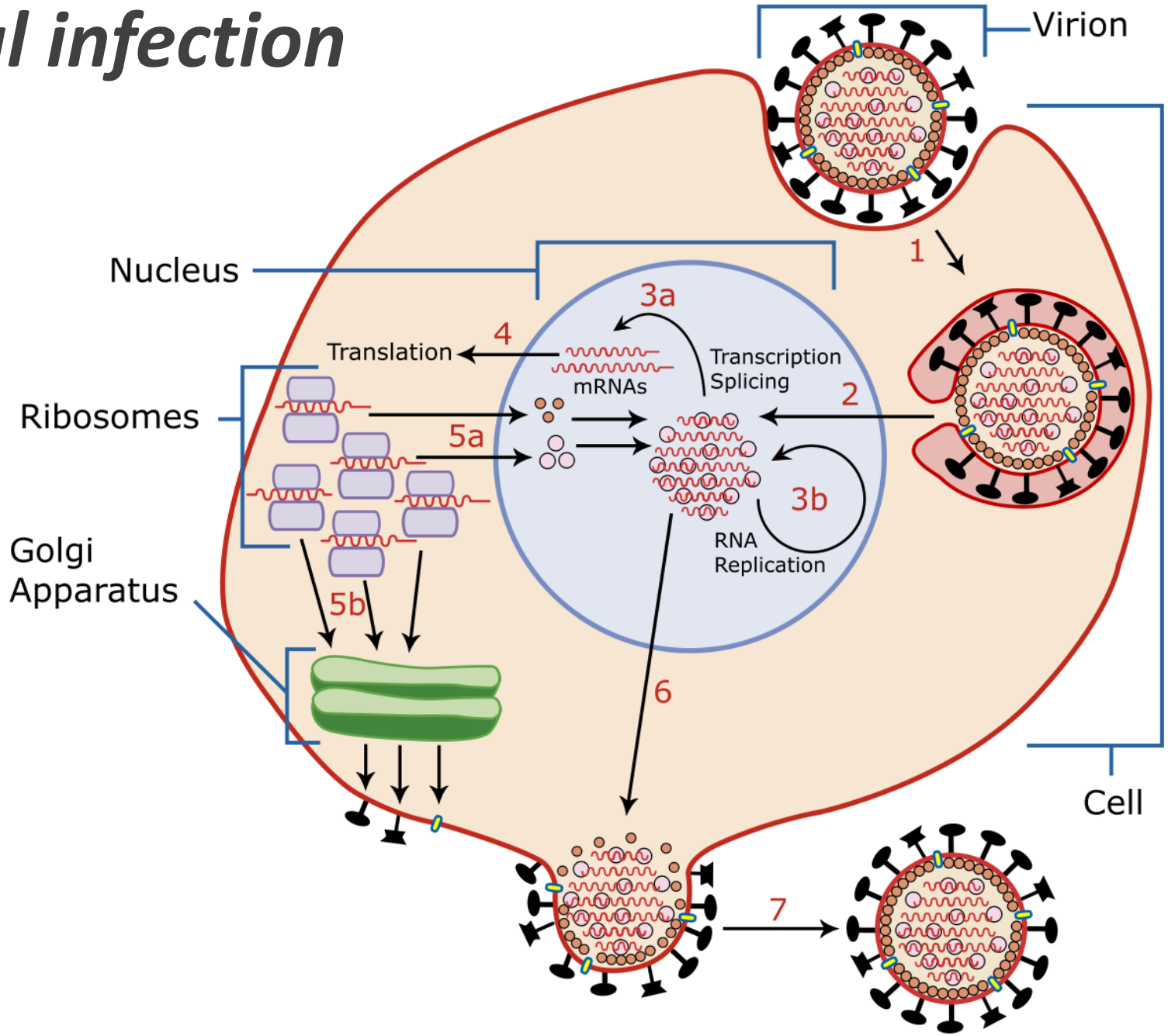
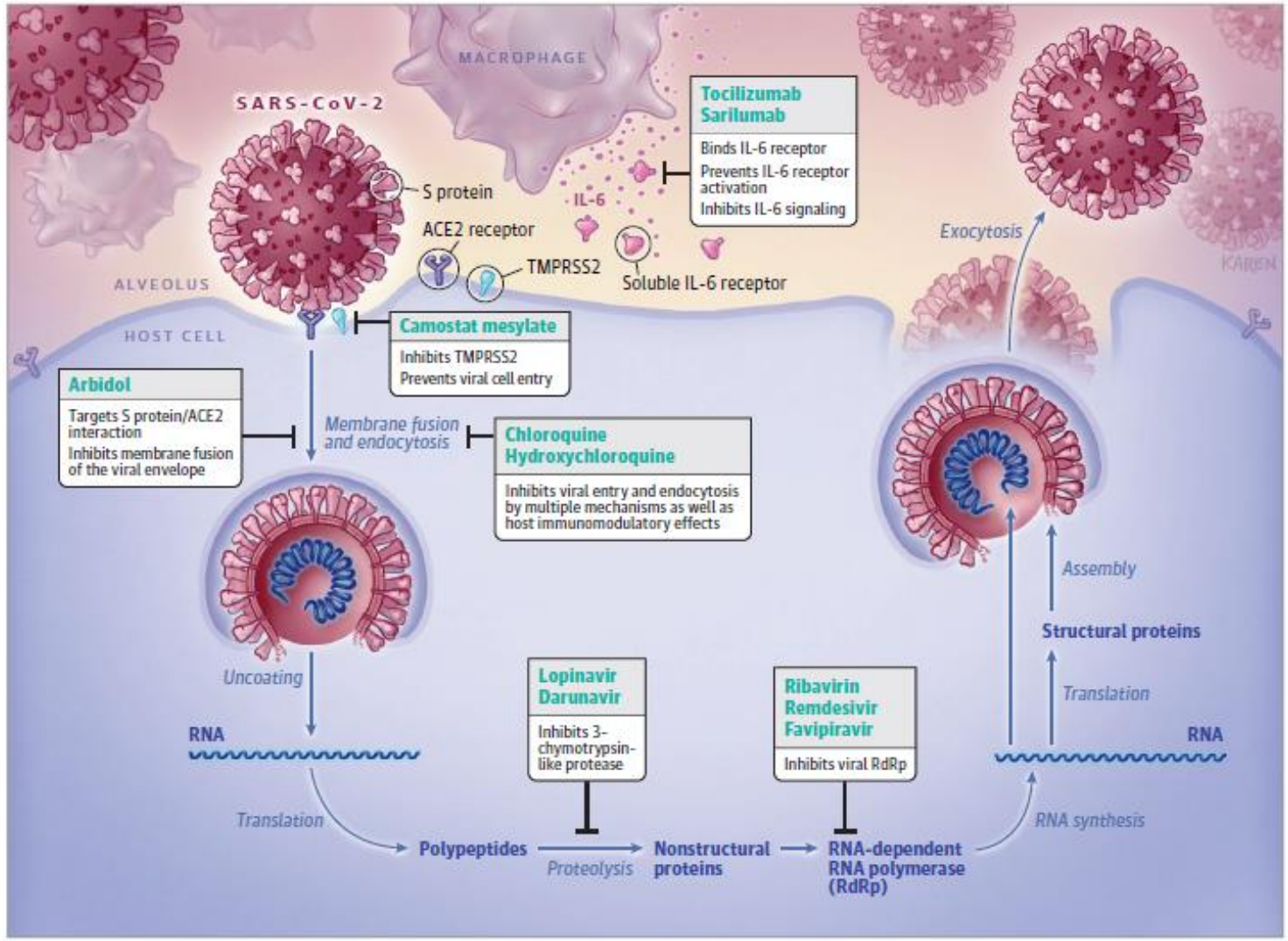


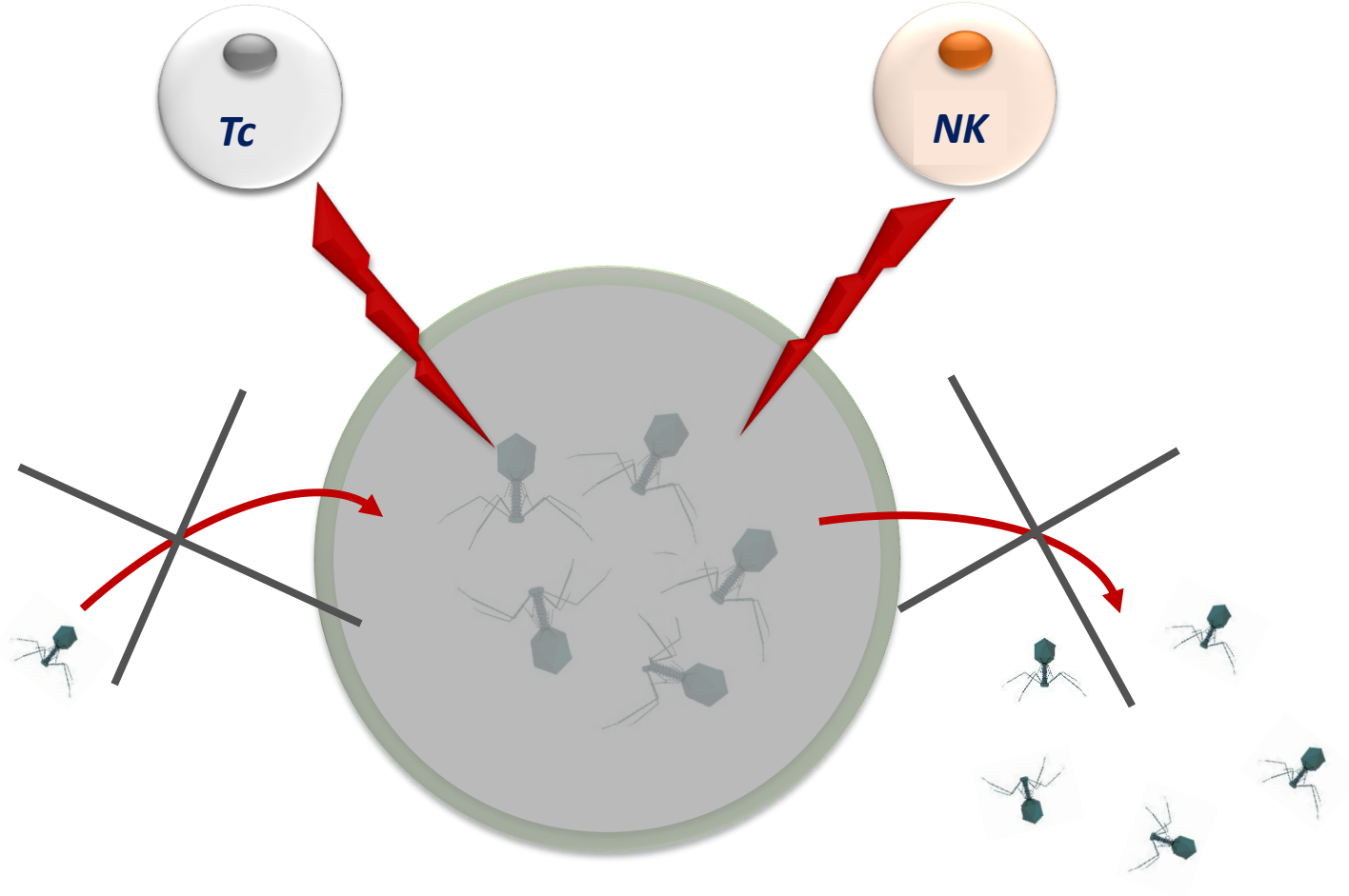
Figure. Simplified Representation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Viral Lifecycle and Potential Drug Targets

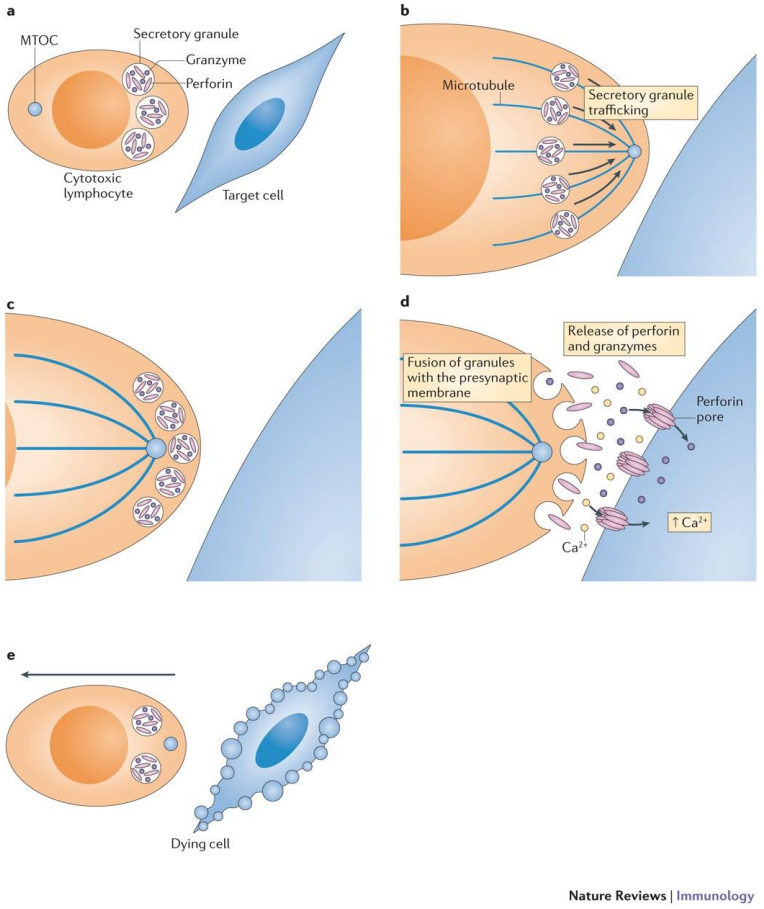


Schematic represents virus-induced host immune system response and viral processing within target cells. Proposed targets of select repurposed and investigational products are noted. ACE2, angiotensin-converting enzyme 2; S protein, spike protein; and TMPRSS2, type 2 transmembrane serine protease.

Everything but the CELL MEDIATED IMMUNE RESPONSE

CYTOTOXIC ACTIVITY OF Tc and NK-cells

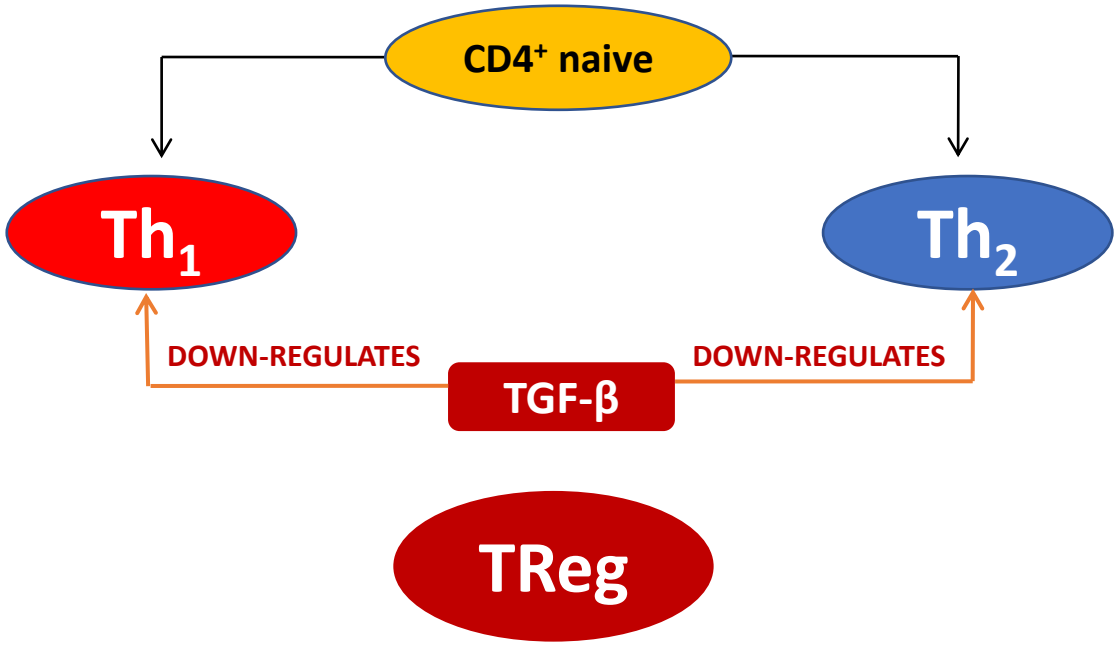




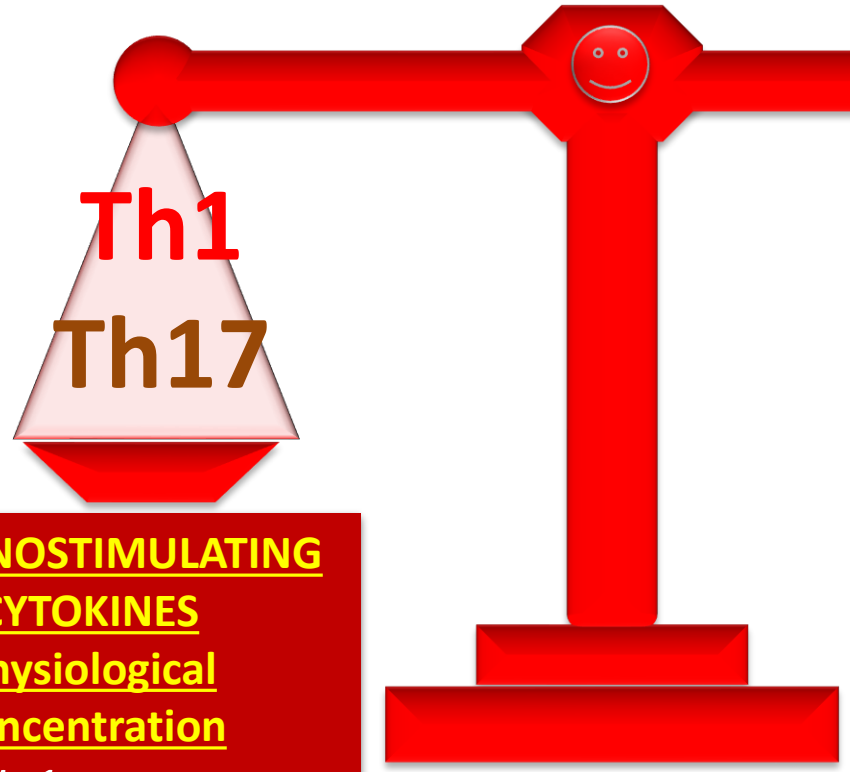
SIGNALING MOLECULES AND DISEASES

DISEASES CAN BE CONSIDERED AS AN EXPRESSION, A CONSEQUENCE OF AN UMBALANCE OF T- HELPER SUBSETS AND CHANGED EXPRESSIONS OF *SIGNALING MOLECULES* CONCENTRATION

Relationship between Th1-Th2-TReg



Relationships between Th subsets



GOOD

IMMUNOSTIMULATING CYTOKINES
Physiological concentration

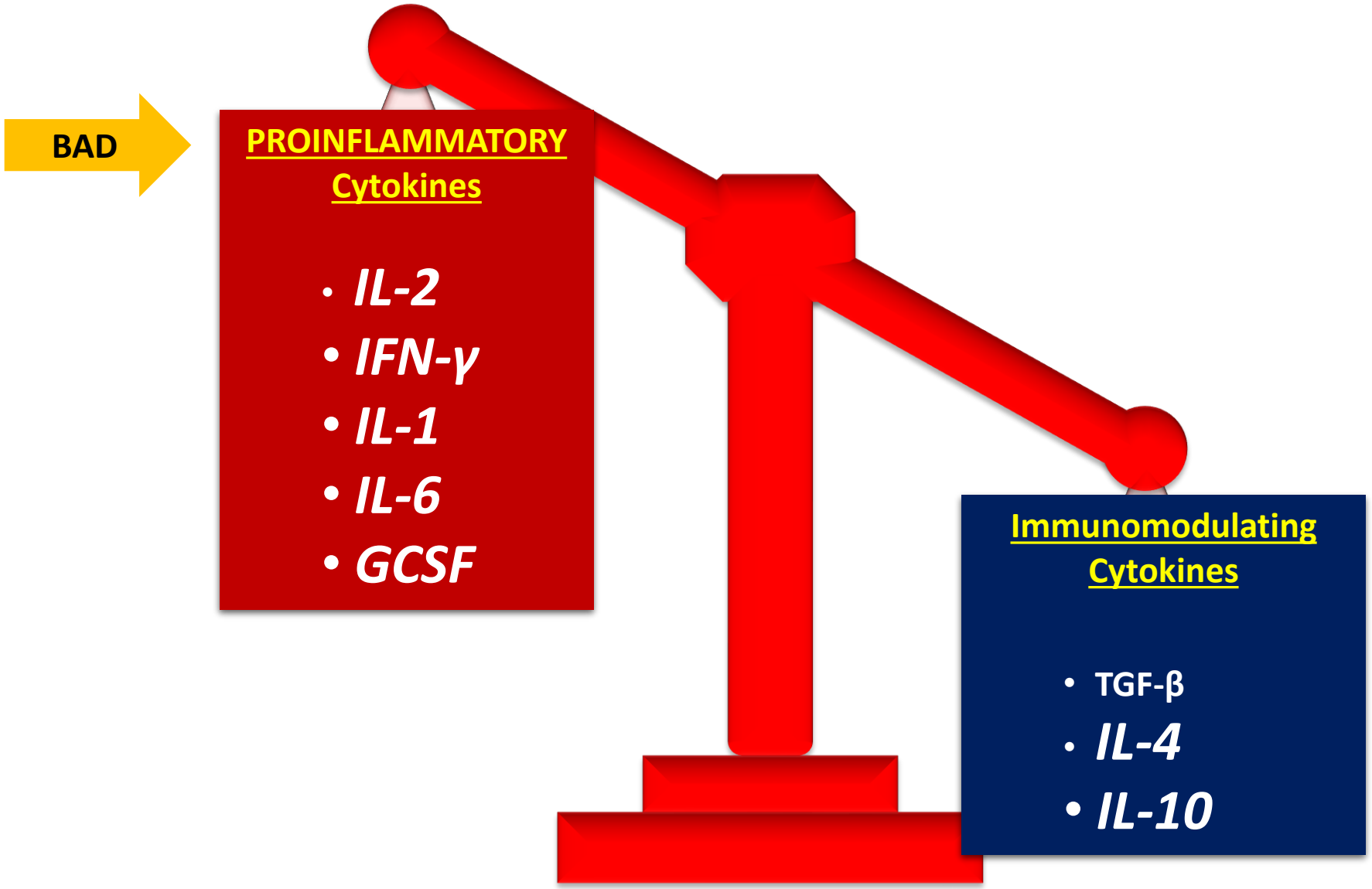
- *IL-1*
- IL-6
- *TNF-α*
- *INF-γ*
- IL-2
- IL-8
- *IL-17*

IMMUNOMODULATING CYTOKINES
Physiological concentration

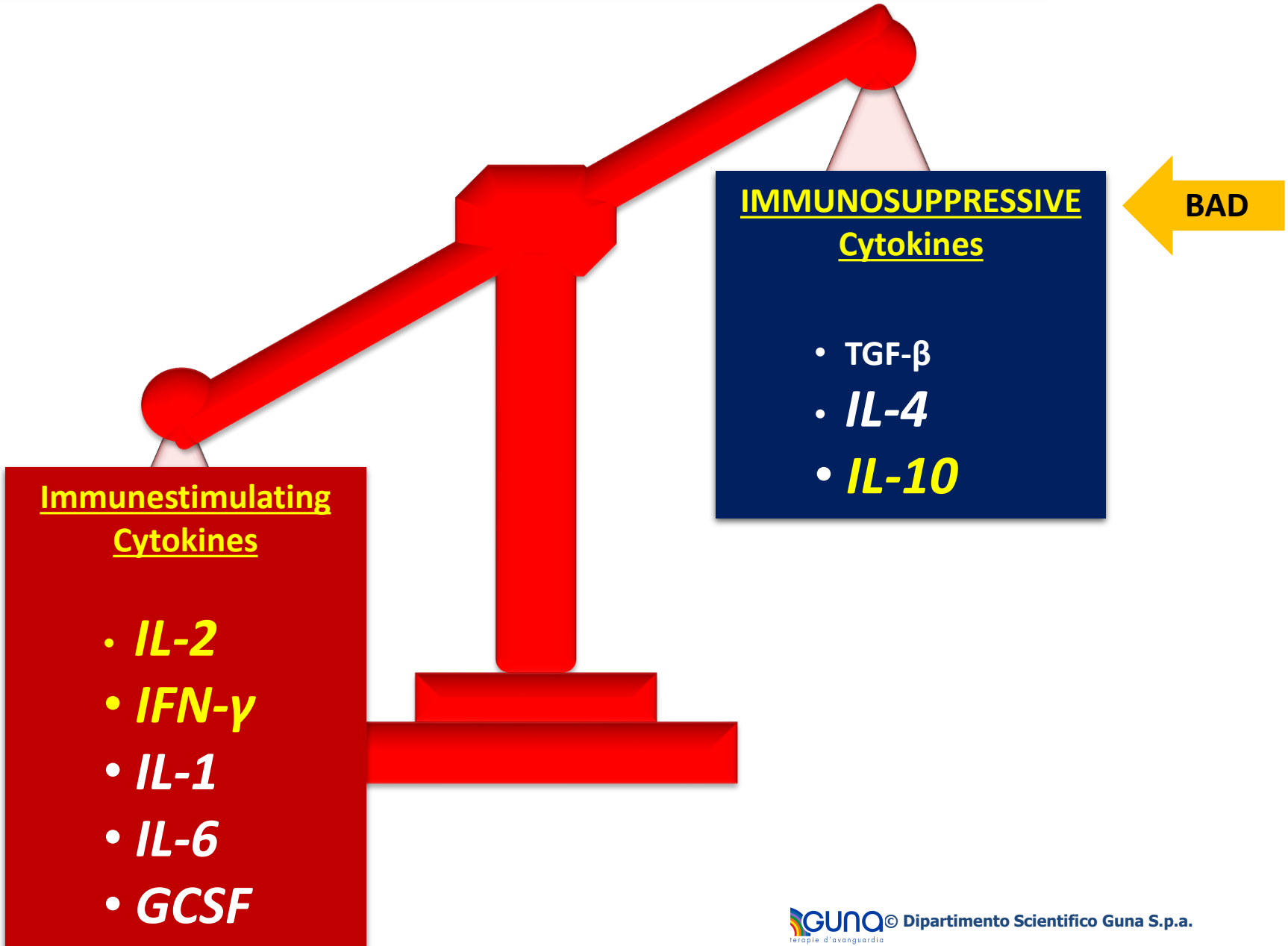
- TGF-β
- *IL-4*
- *IL-10*

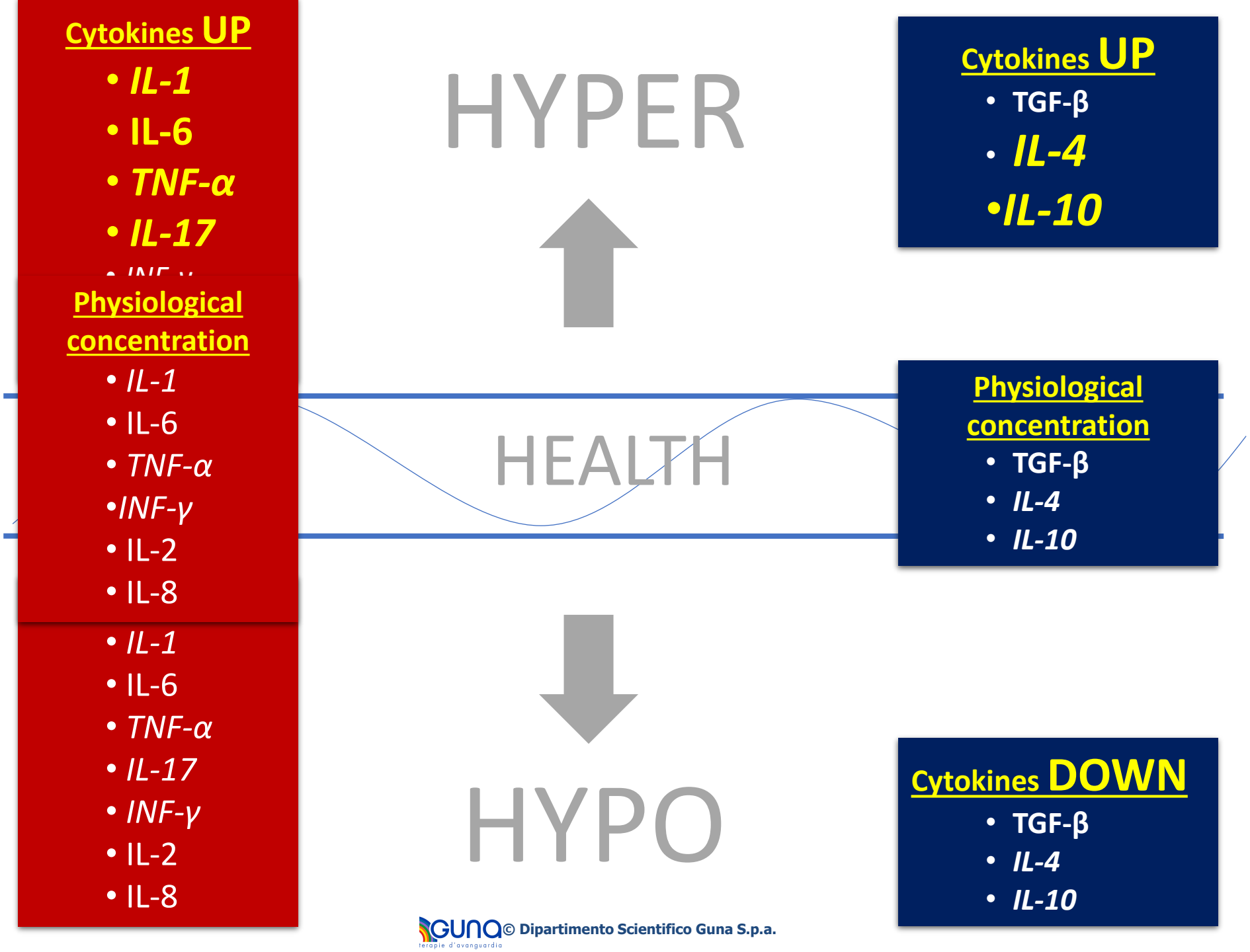
GOOD

...in inflammation

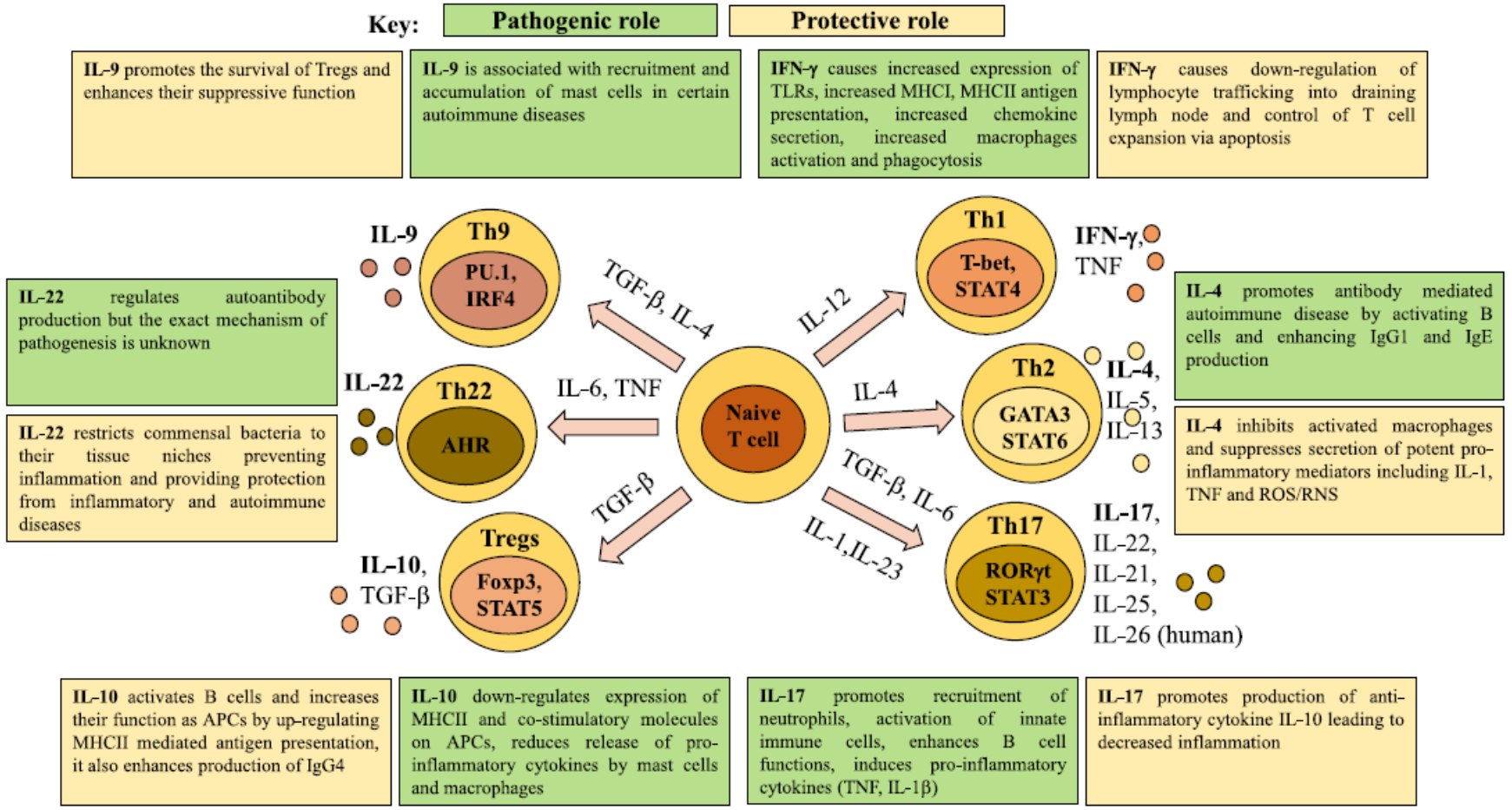


…in Immune “deficiency”





Neither good nor bad in Nature



Raphael I et al. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine (2014), http://dx.doi.org/10.1016/j.cyto.2014.09.011

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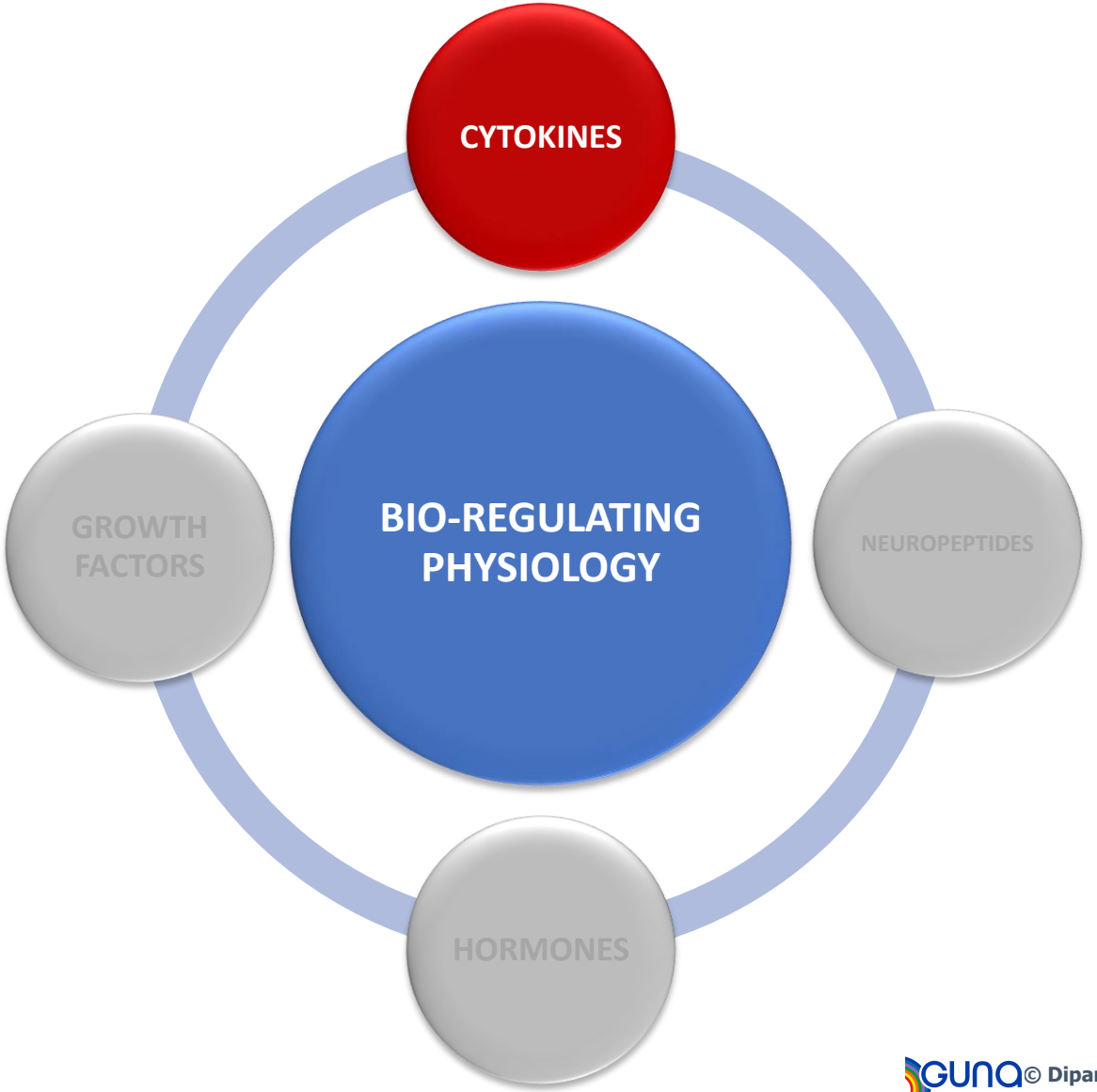
Clinical use of low dose SKA signaling molecules

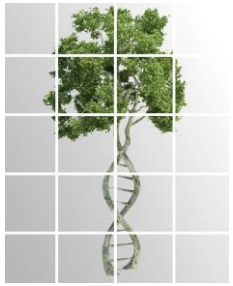
THE SCALES OF THE BODY

LOW DOSE SIGNALING MOLECULES

THE GREAT INNOVATION



GUNA Signaling Molecules



- Bio-Tech – human recombinant in *E. Coli or in SF21 (Spodoptera frugiperda); for Anti IL-1 in mouse.*

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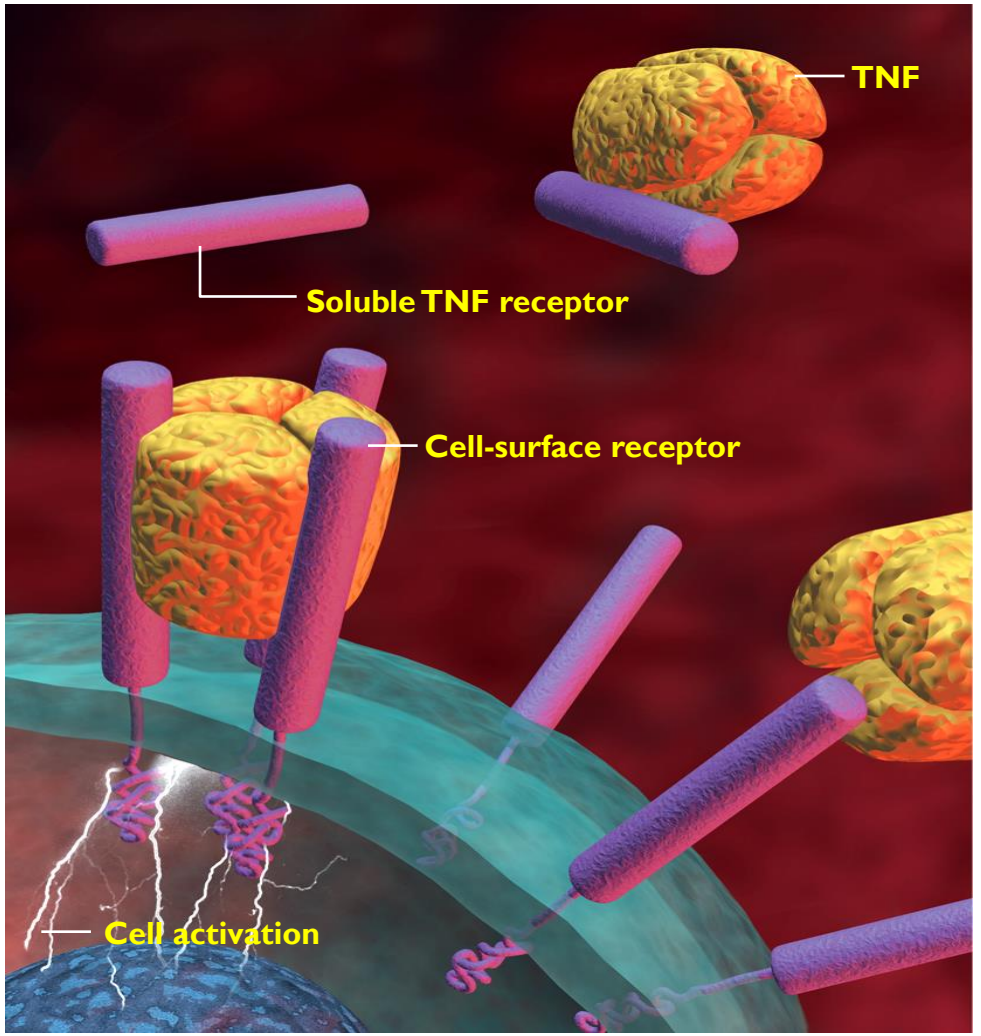
TRANS-MEMBRANE RECEPTORS
Up- and Down-Regulation



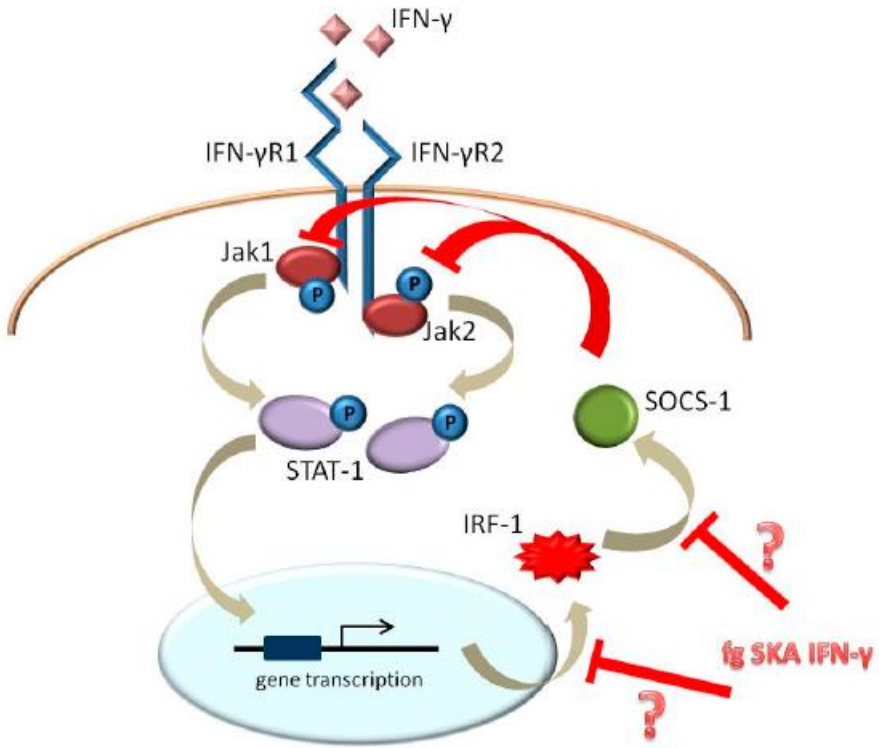
International Journal of
Molecular Sciences

MDPI

Article

Femtograms of Interferon-γ Suffice to Modulate the Behavior of Jurkat Cells: A New Light in Immunomodulation

Sara Castiglioni [1,*], Vincenzo Miranda [2], Alessandra Cazzaniga [1], Marilena Campanella [2], Michele Nichelatti [3], Marco Andena [1] and Jeanette A. M. Maier [1]



Jak-1: Tyrosine kinasis
STAT-1: Signal transducer and activator of transcription 1
SOCS-1:Suppressor of cytokin signaling 1

Safety and biological "INTELLIGENCE" of LOW DOSES

Journal of Cancer Therapy, 2012, 3, ***-***
Published Online September 2012 (http://www.SciRP.org/journal/jct)

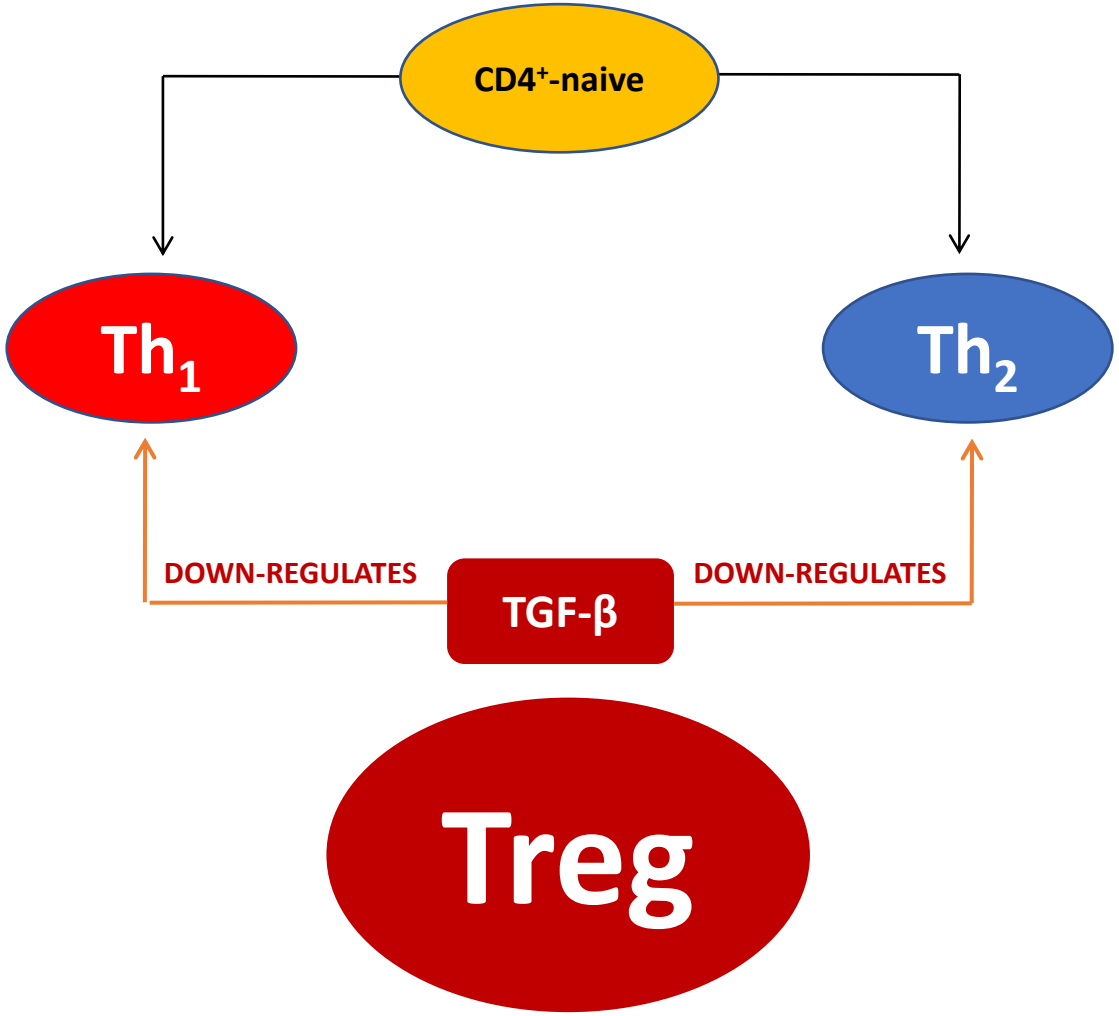
Low Dose of IL-12 Stimulates T Cell Response in Cultures of PBMCs Derived from Non Small Cell Lung Cancer Patients*

Lucia D'Amico[1], Enrico Ruffini[2], Riccardo Ferracini[3], Ilaria Roato[1#]

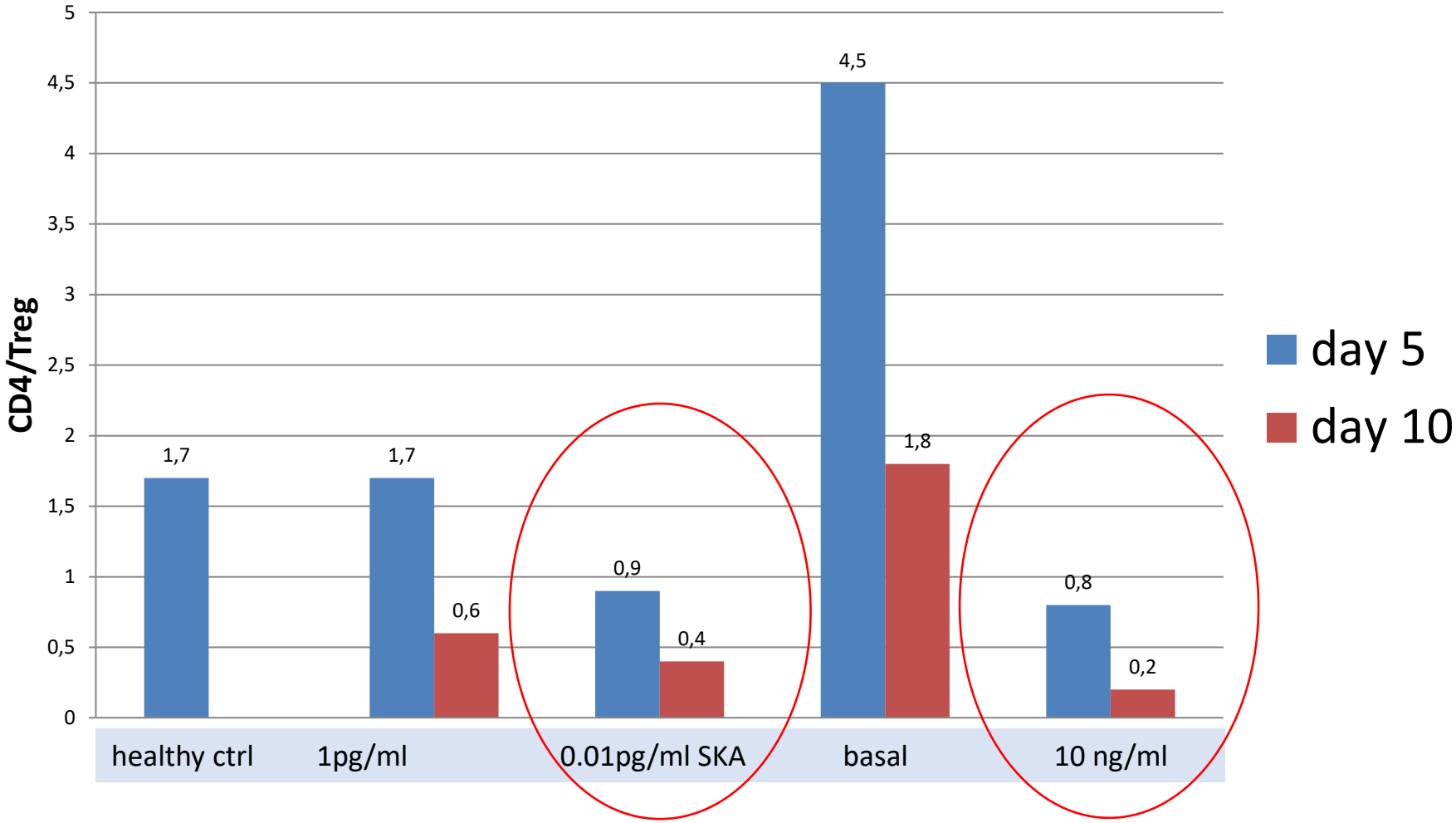
[1]CeRMS (Center for Research and Medical Studies), A.O. della Salute e della Scienza di Torino, Torino, Italy; [2]Department of Toracic Surgery, A.O. della Salute e della Scienza di Torino, Torino, Italy; [3]Department of Orthopaedics, A.O. della Salute e della Scienza di Torino, Torino, Italy.
Email: #roato78@libero.it

Received 2012

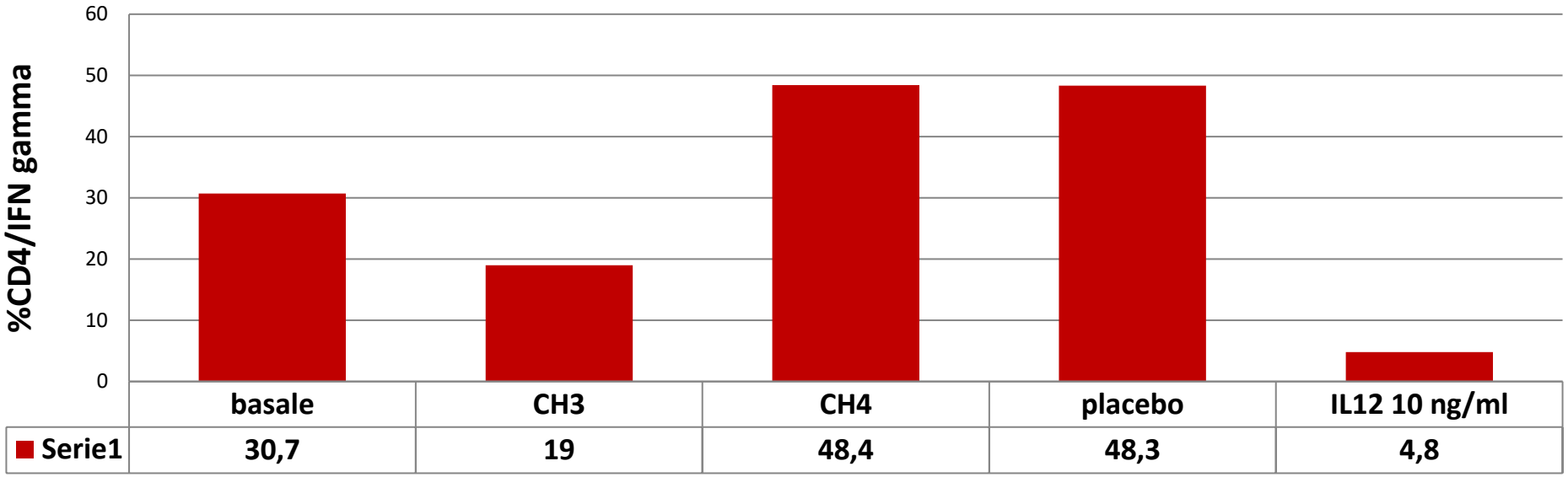
Relationship among Th1-Th2-Treg



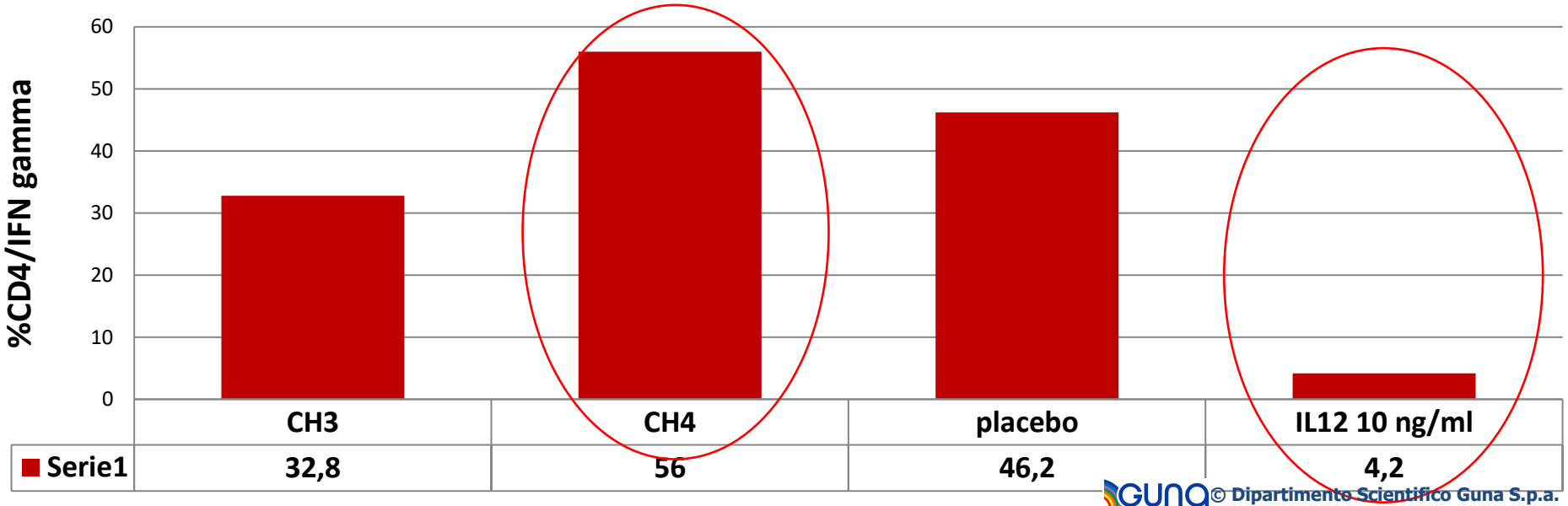
Safety of low dose cytokines



Day 5



Day 10



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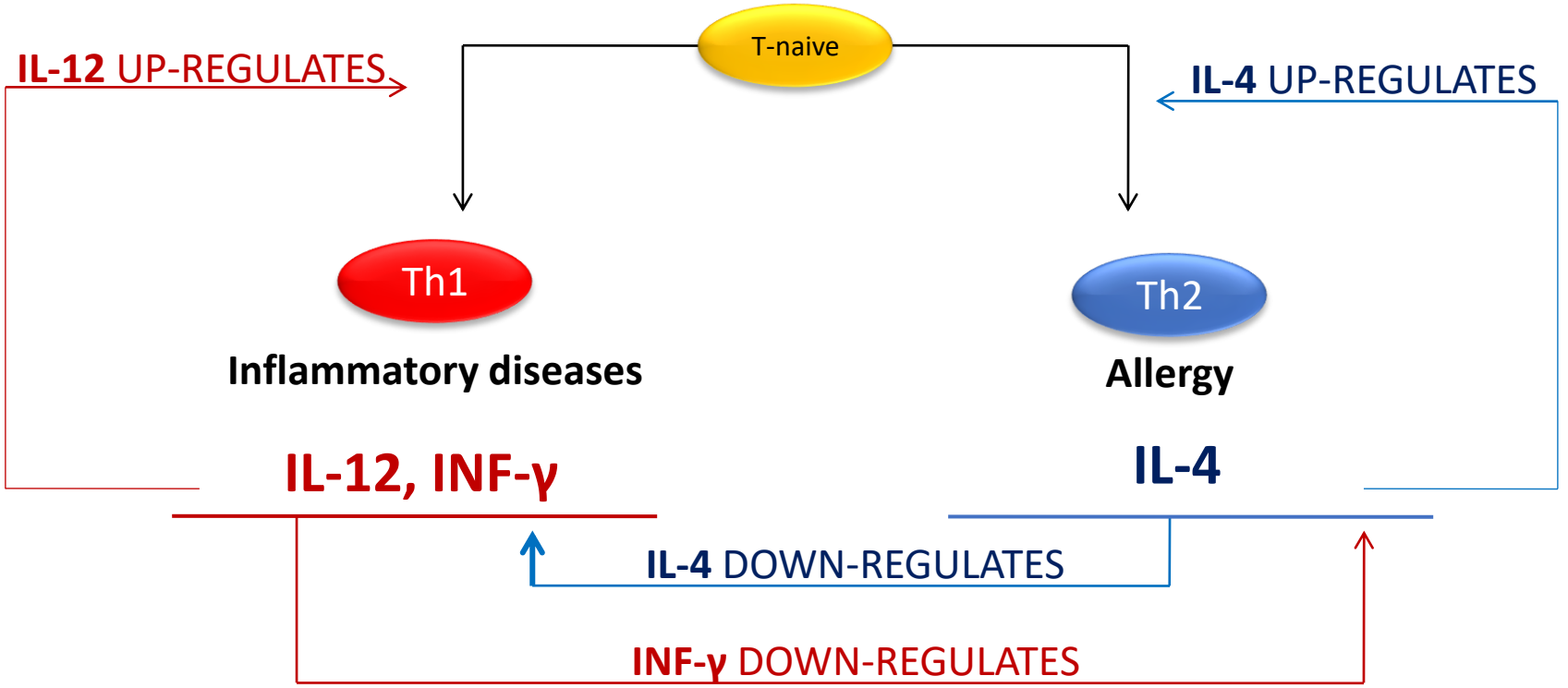
*The concept of **BALANCE** and the use of SKA low dose cytokines*

Antagonistic cytokines are utilized in order to slow down a biological effect; **Same cytokines** in order to enhance a biological function.

THE CONCEPT OF BALANCE – RECIPROCITY of TH CELLS



Th subsets cross-regulate expansion and functions each other.

- *Cooke A. Th17 in Inflammatory Conditions.* ***2006, Rev Diabetic Stud 3: 72-7***
- *Bettelli E. et al. Th17: the third member of the effector T cell trilogy.* ***Current Opinion in Immunology 2007, 19: 652-657***

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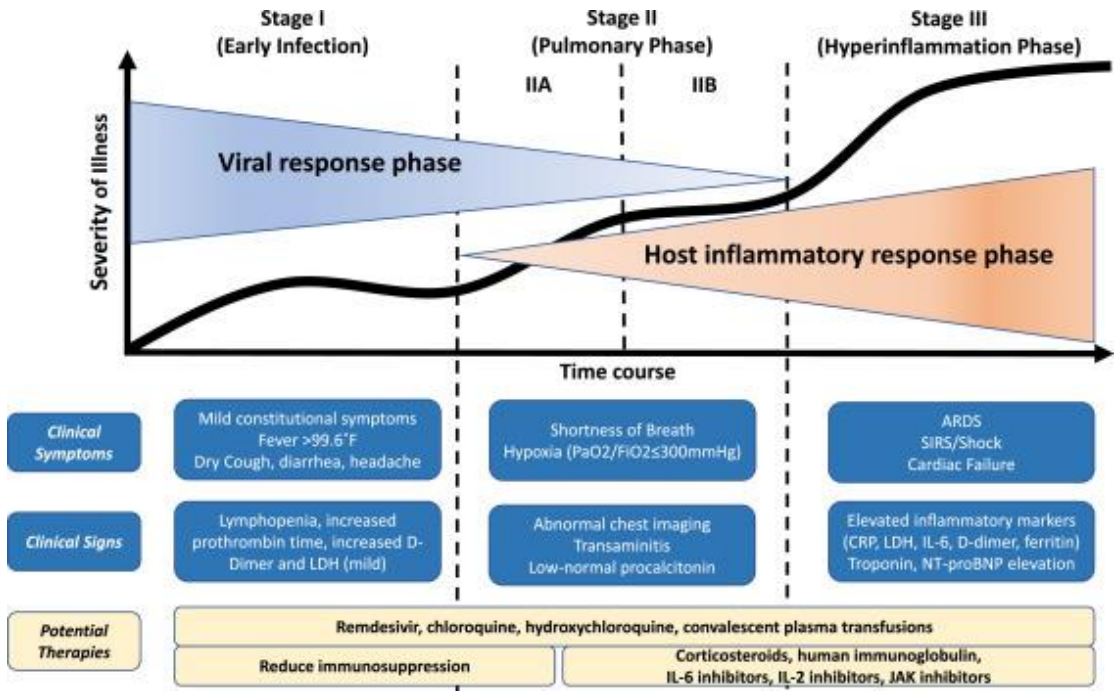
PRESCRIPTION ACCORDING TO THE AETIOLOGICAL DECISIONAL PROCESS

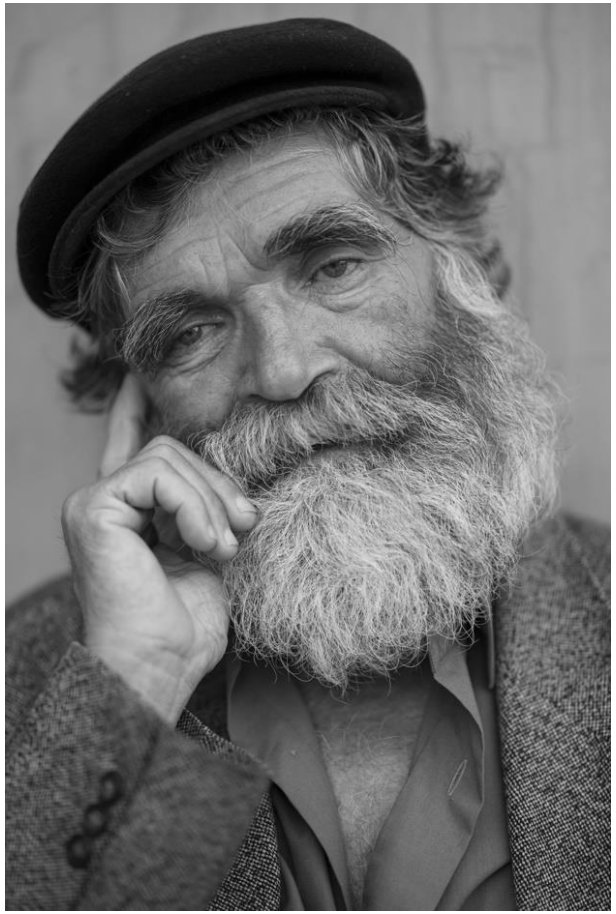
CYTOKINE	STRENGTHENING same cytokine	MODULATION opposing cytokine
GCSF	GCSF 4C	IL-10 4C/IL-4 4C
INF alpha/gamma	INF alpha/gamma 4C	IL-4 4C
IL-1	IL-1 4C	Guna Anti IL-1 4C/IL-10 4C
IL-2	IL-2 4C	IL-11 4C
IL-3	IL-3 4C	IL-10 4C
IL-4	IL-4 4C	INF-gamma 4C/IL-12 4C
IL-5	IL-5 4C	TGF-beta 4C
IL-6	IL-6 4C	IL-10 4C
IL-7	IL-7 4C	IL-10 4C/TGF-β1 4C
IL-8	IL-8 4C	IL-10 4C/TGF-β1 4C
IL-9	IL-9 4C	IL-10 4C
IL-10	IL-10 4C	IL-1 4C/TNF 4C/IL-6 4C
IL-11	IL-11 4C	IL-2 4C
IL-12	IL-12 4C	IL-4 4C/IL-10 4C
TGF-beta 1	TGF-beta 4C	IL-12 4C
TNF	TNF-alpha 4C	Guna Anti IL-1 4C+IL-10 4C

Our (unique) goal

- ***Before***
- *During*
- *After*

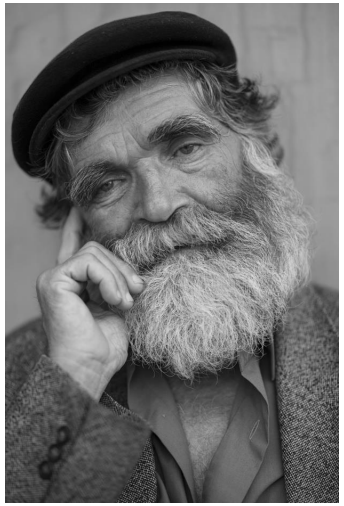


Elderly people and kids

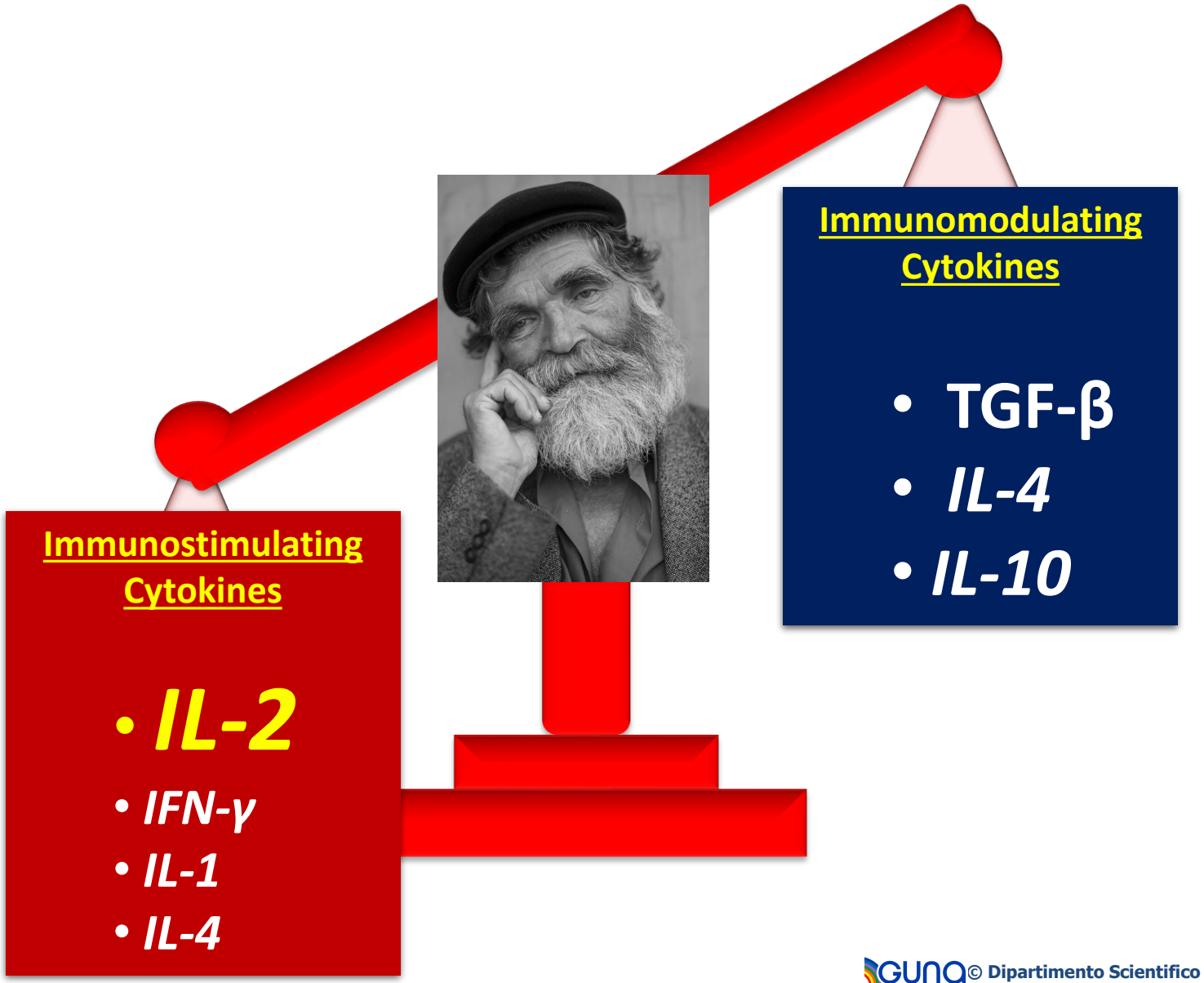


Immune-aging and Immune-decline

Modulation of the immune response



…in age-related Immune "Decline"



IL-2/IL-6 RATIO AND AGING

INCREASING OF:

- Coagulation factors
- Homocysteine
- IL6
- Proinflammatory cytokines
- Acute Phase Proteins
- Stress hormones
- ROS
- Lp(a)

A PROINFLAMMATORY STATUS OF HEALTHY ELDERLY AND CENTENARIANS

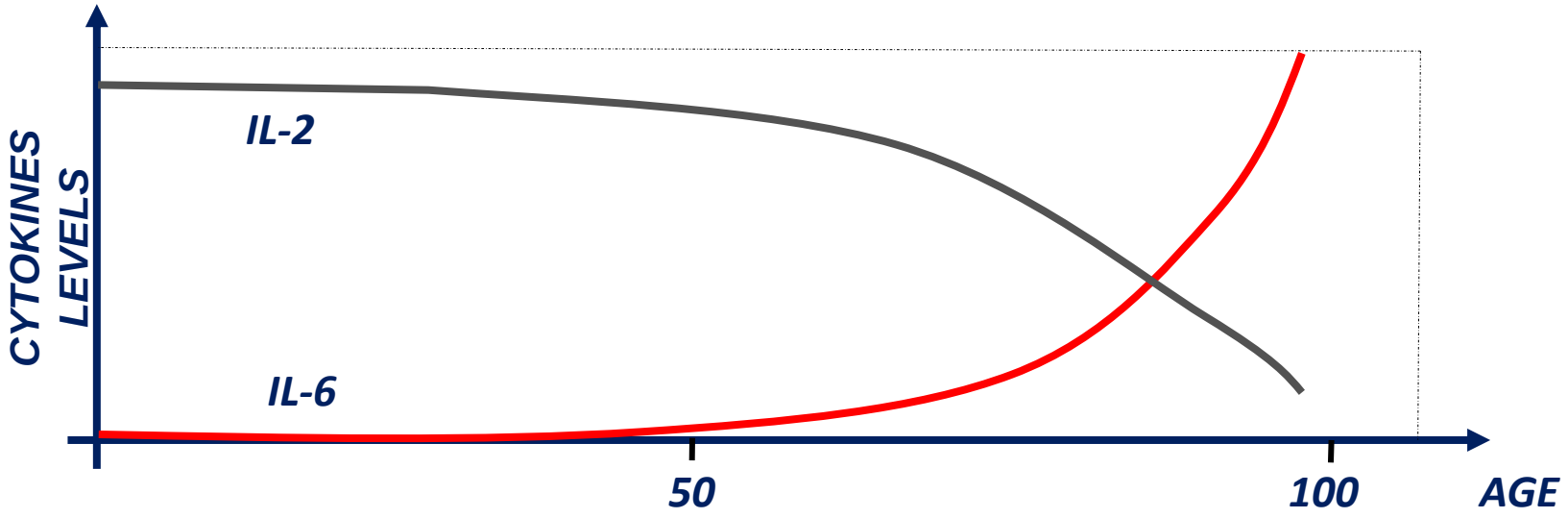
ELSEVIER

Mechanisms of Ageing and Development
100 (1998) 313–328

mechanisms of ageing and development

Increase of interleukin 6 and decrease of interleukin 2 production during the ageing process are influenced by the health status

Jolanta Myśliwska [a,*], Ewa Bryl [a], Jerzy Foerster [b], Andrzej Myśliwski [a]

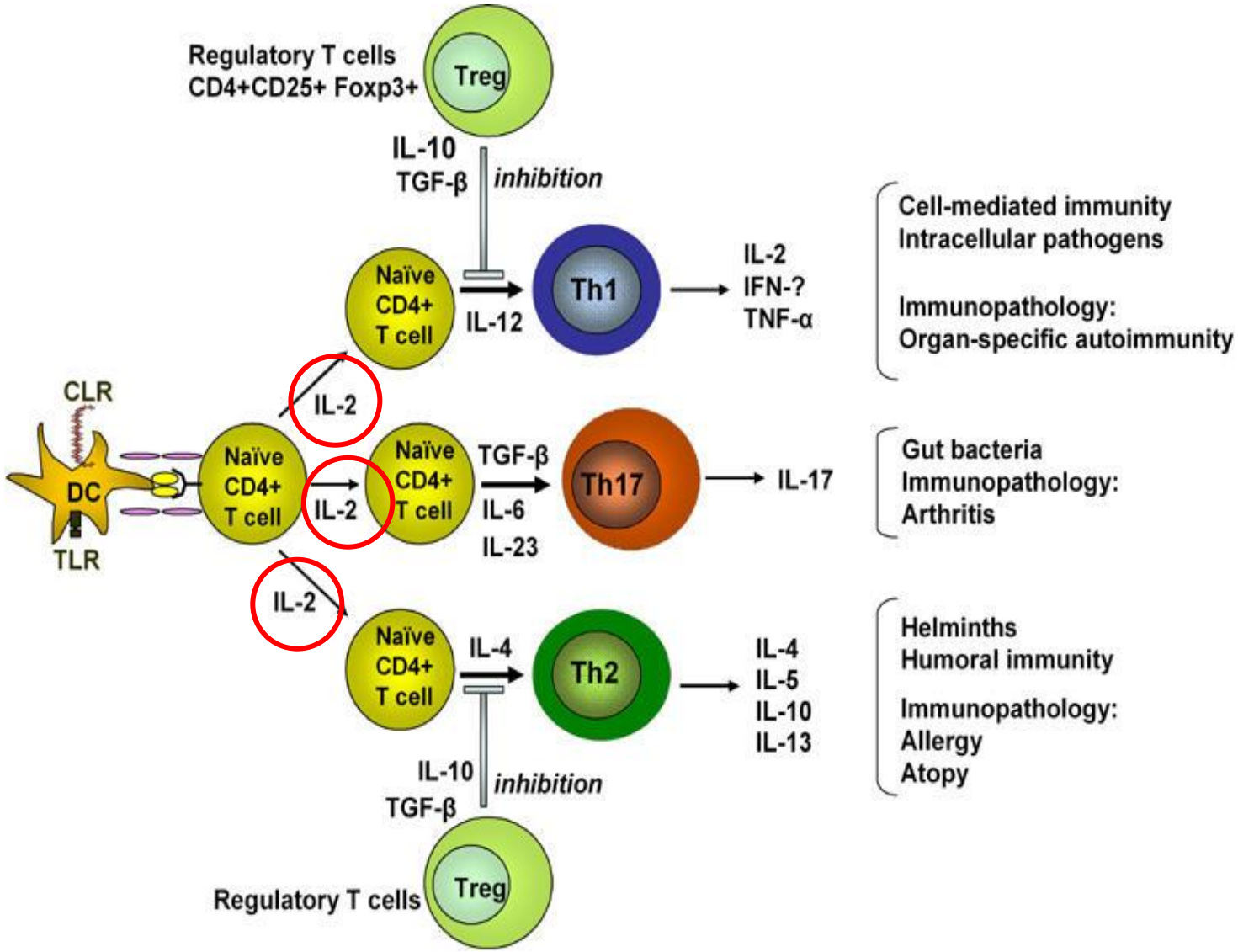


IMMUNE-AGING AND IMMUNE-DECLINE

Much of immune decline may be traceable to a decreased ability of activated ***T-cells*** *(both helpers and cytotoxic cells)* ***to undergo clonal expansion***

Such an expansion has an absolute dependence on INTERLEUKIN-2 (IL-2) activity

THE PIVOTAL ROLE OF INTERLEUKIN-2



Antigen presentation to naïve T cells results in the development of Th1, Th2 or Th17 cells depending on the cytokine milieu.

From: **The Review of Diabetic Studies (2006) 3:72-75**

A novel, systemic, approach to
Aspecific immunostimulation and immune decline-related disorders

DIRECTIONS AND WAY OF ADMINISTRATION

- **Guna-Interleukin 2: 20 drops twice a day for 2 to 4 months (half dosage for children below 6 years)**

Sublingual administration directly under the tongue or in a little water, preferibly far from meals.

INTERLEUKIN-2 INDUCES THE CLONAL EXPANSION OF T LYMPHOCYTES

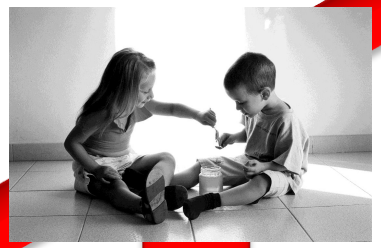
- **Interleukin-2 (IL-2)**, identified more than 40 years ago, **was initially called T Cell Growth Factor**; it induces the T lymphocytes to enter the S phase of the cell cycle, favoring their expansion. From the outset, its fundamental role in the management of the immune response and the pharmacological potential associated with it was evident.

- **IL-2** is produced by activated T lymphocytes and has a key role in triggering immune responses. **The main effect of IL-2 is to induce the clonal expansion of T lymphocytes after antigen recognition; moreover, IL-2 induces the proliferation of activated B lymphocytes, increases the levels of Natural Killer (NK) cells, supports cytotoxicity mediated by T Lymphocytes (CTL - Cytotoxic T-lymphocytes), stimulates the production of other cytokines including TNF, IFN-γ and GM-CSF.**

Immunodeficiency in childhood

Modulation of the immune response

In childhood immunodeficiency…



Immunomodulating Cytokines

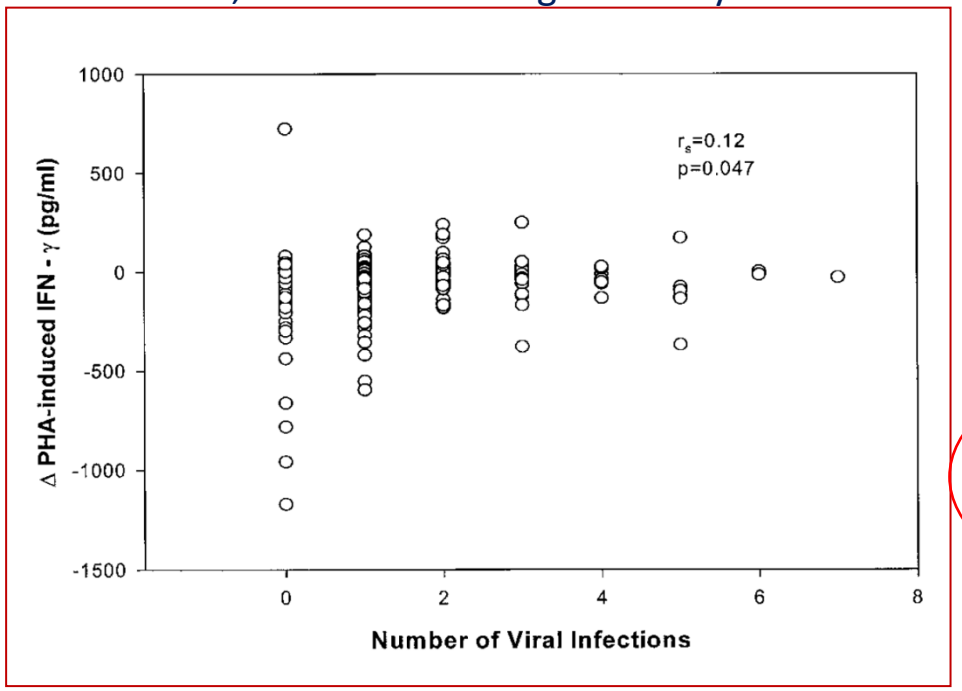
- TGF-β
- *IL-4*
- *IL-10*

Immunostimulating Cytokines

- *IL-2*
- ***IFN-γ***
- *IL-1*
- *IL-6*
- *GCSF*

Cytokines response pattern, viruses exposure and respiratory infections during the first year of life

285 children, monitored during the first year of life



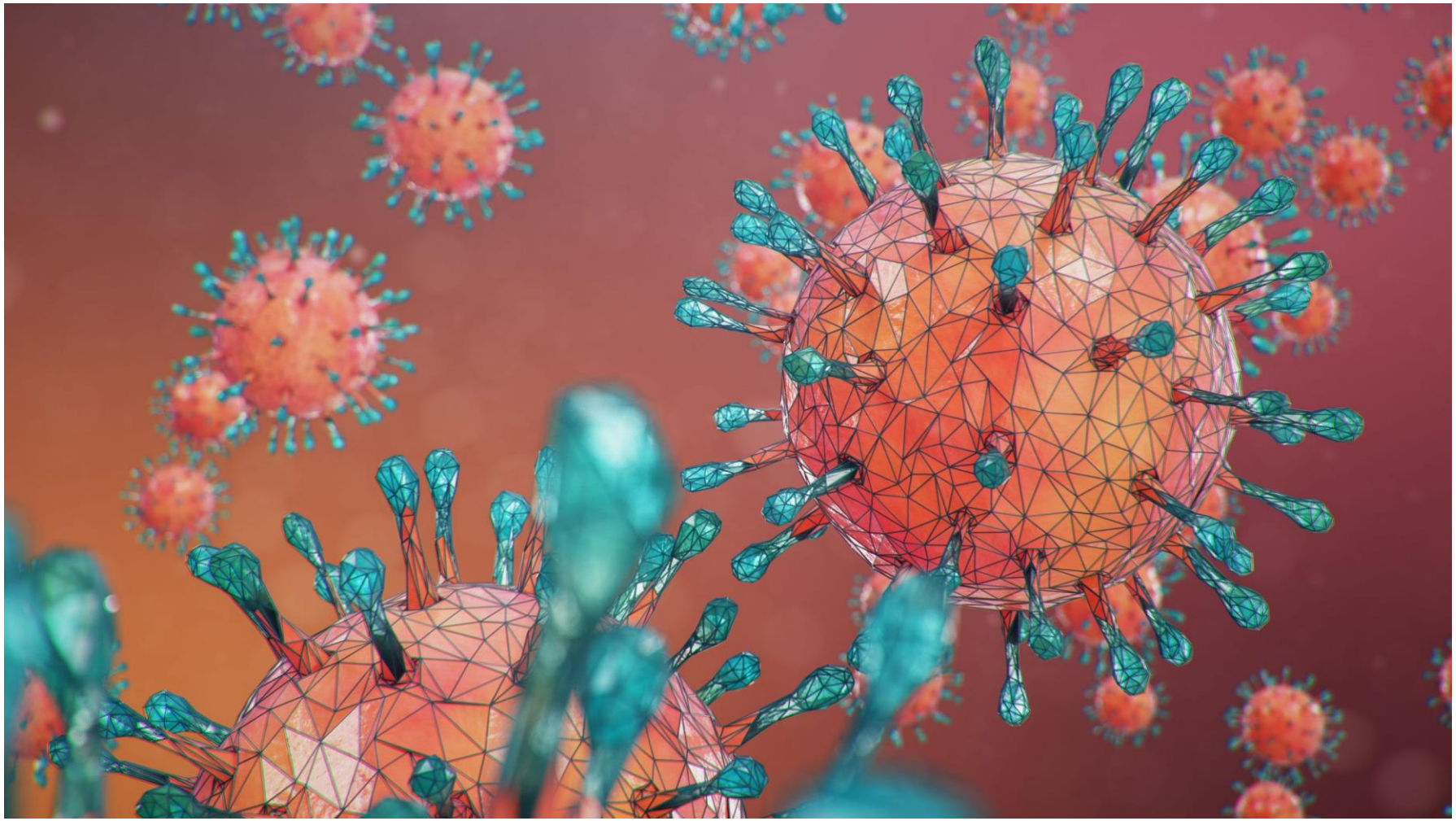
Reduced production of IFN-gamma in the first year of life (57–26 pg/ml, p 0.001)

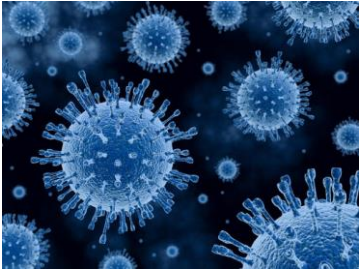
Significant positive correlation between number of respiratory infections and reduced production of **IFN-gamma** (rs 0.12, p 0.047)

Am J Respir Crit Care Med Vol 170. pp 175–180, 2004

Courtesy from Prof. G. Marseglia - Pavia

When do viruses have a party?





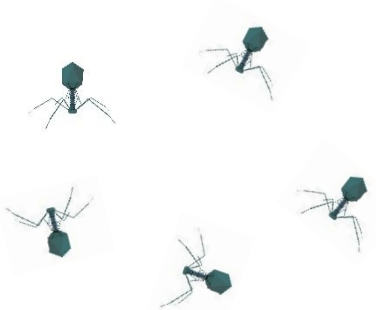
…under viral attack

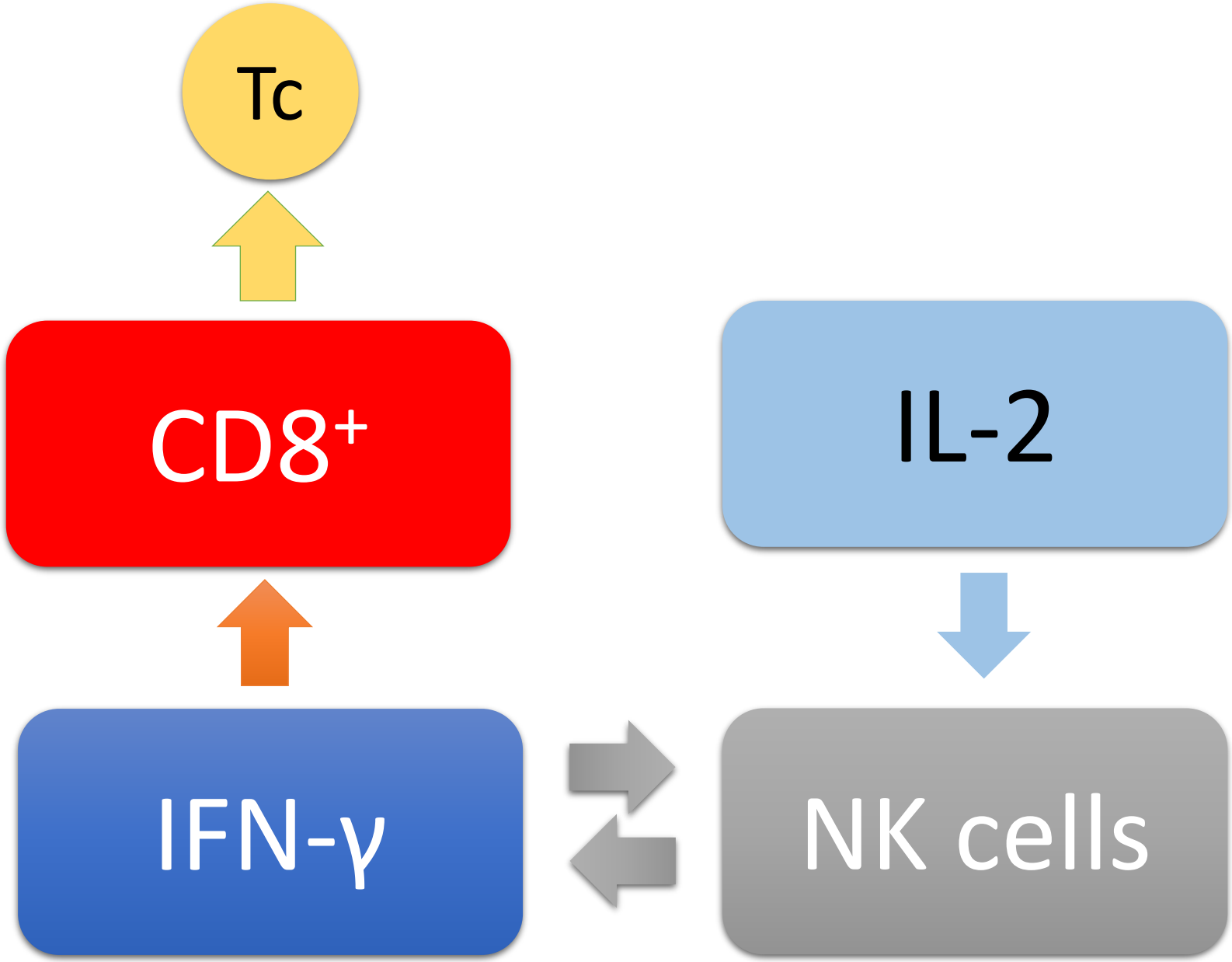
Immunemodulating Cytokines

- TGF-β
- *IL-4*
- *IL-10*

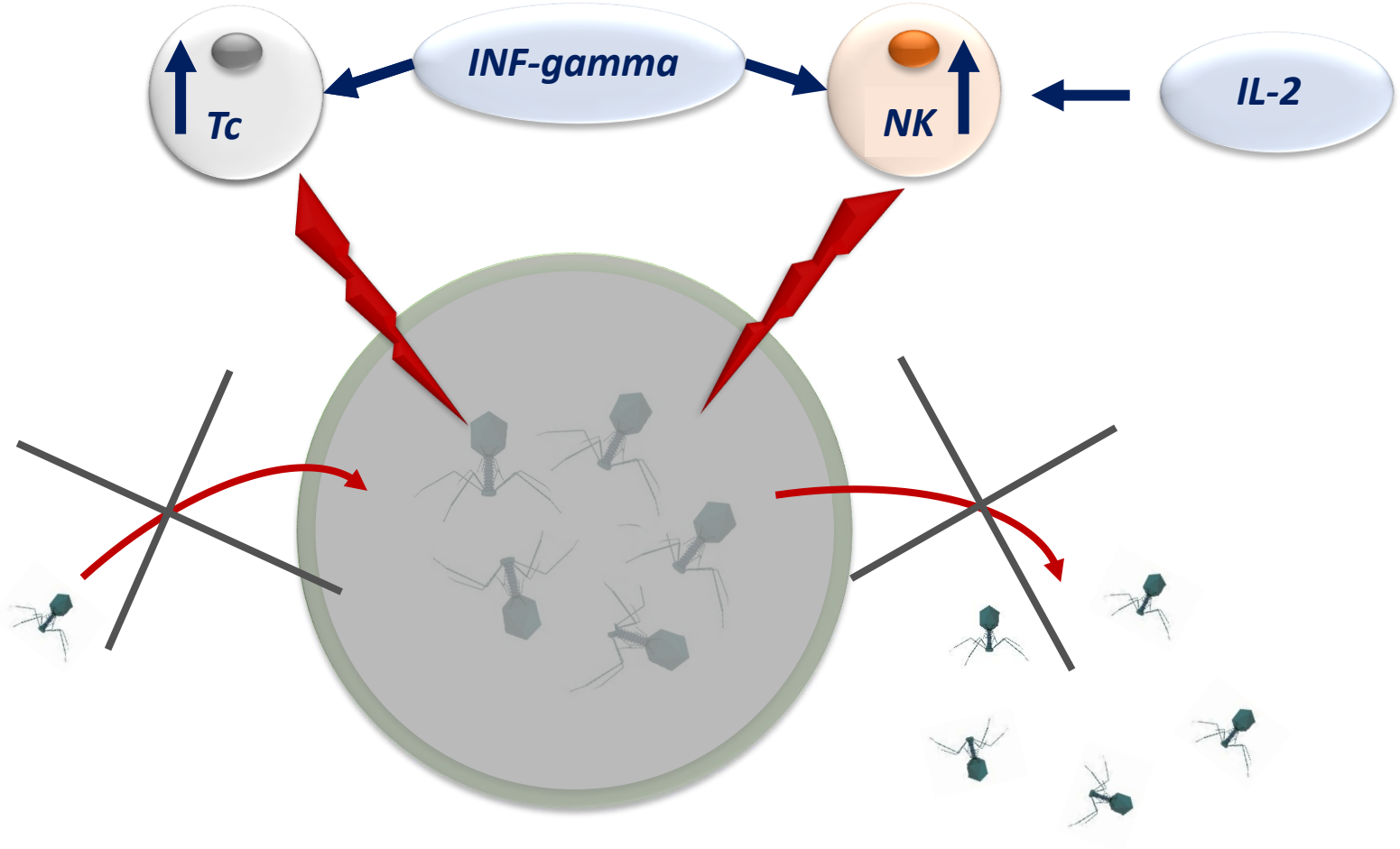
Immunestimulating Cytokines

- *IFN-γ*
- *IL-2*
- *IL-1*
- *IL-6*
- *GCSF*



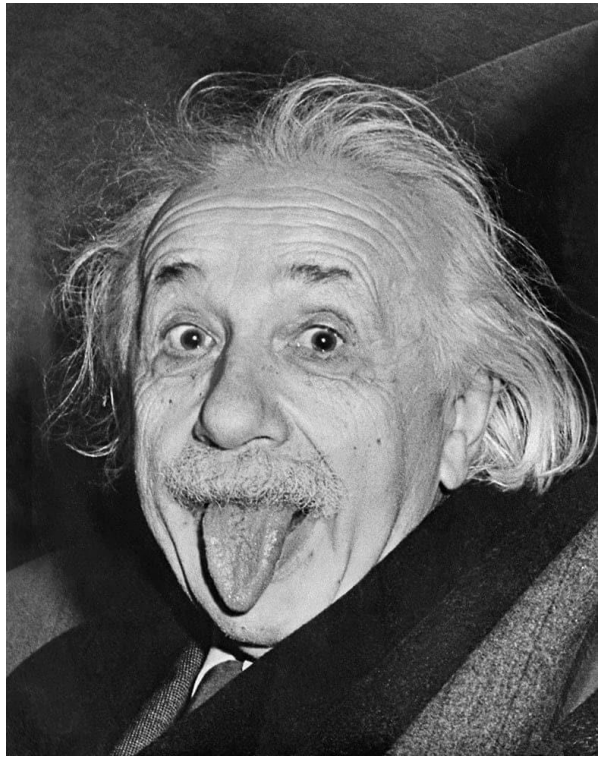


CYTOTOXIC ACTIVITY OF IFN-gamma

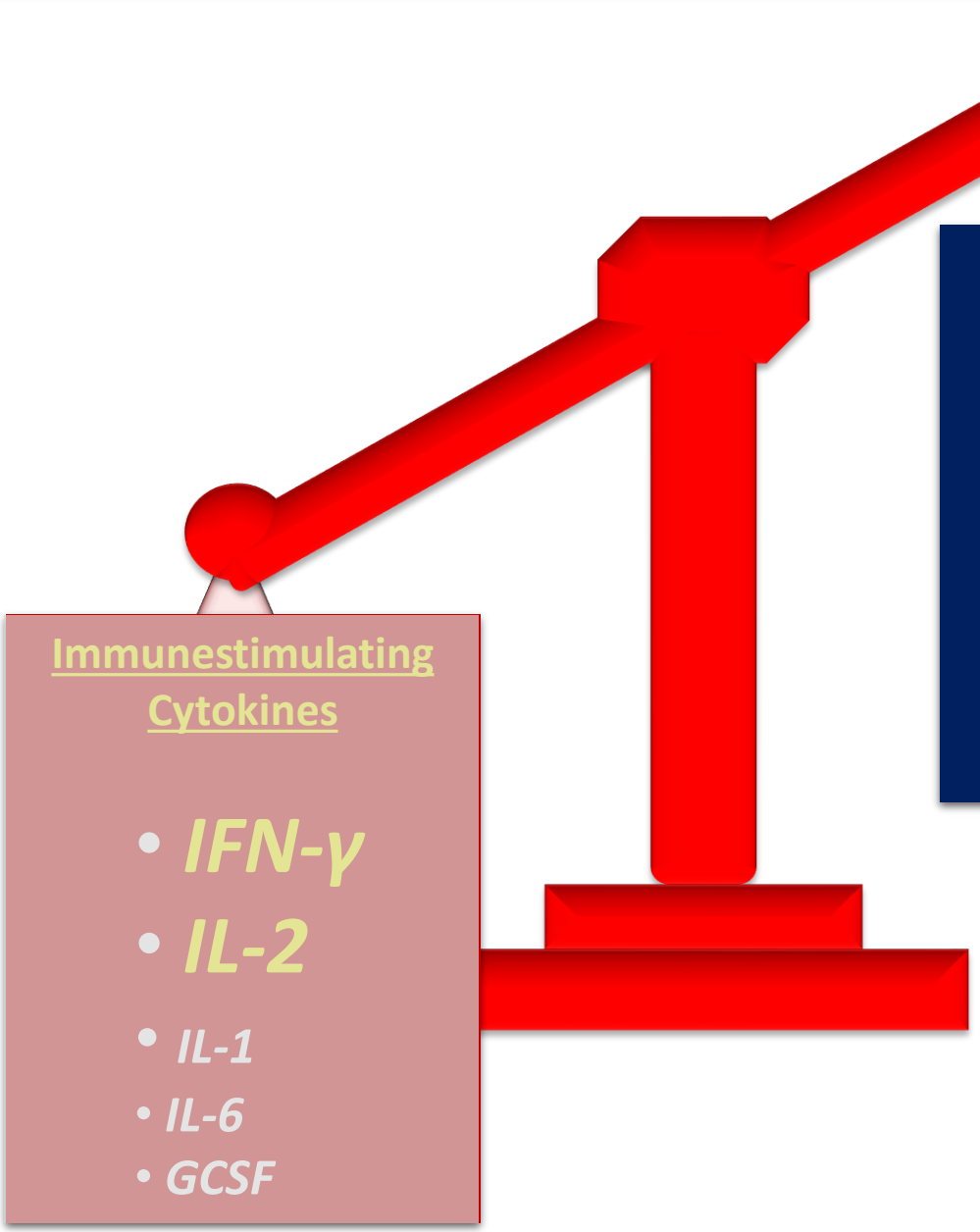


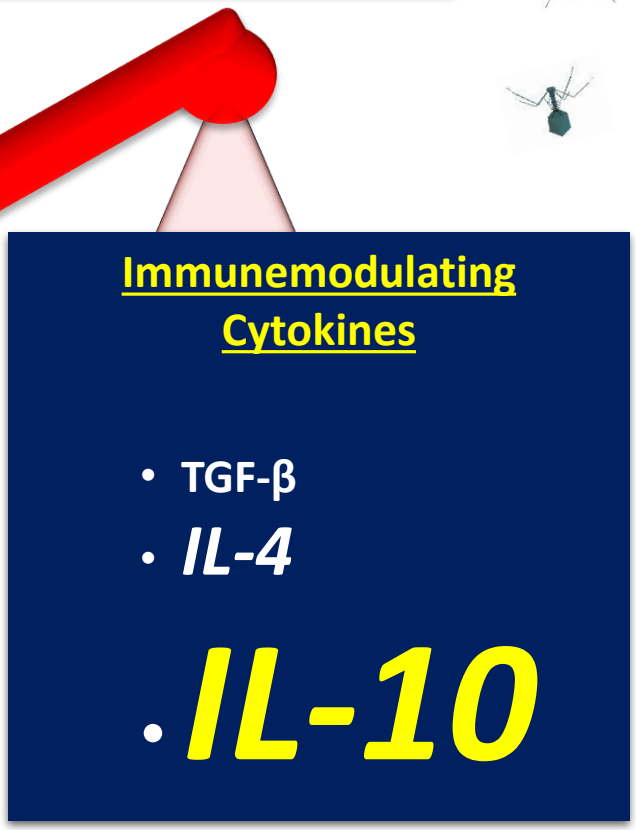
guna.it

Viruses are super smart



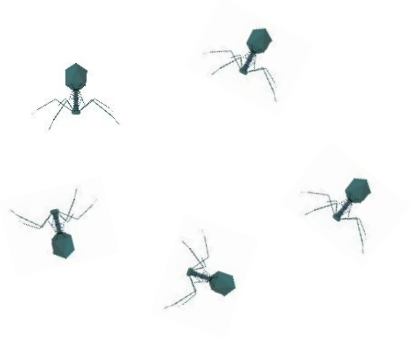
…under viral attack



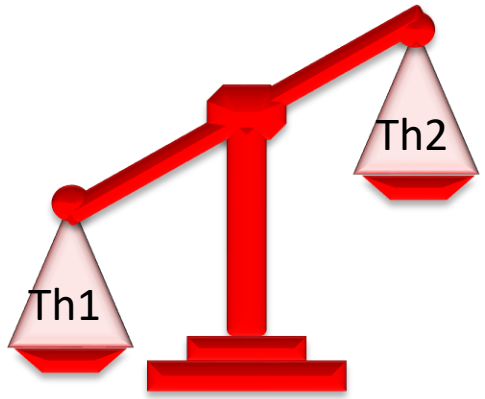


Virus immune-escape mechanisms

Viral infection and cell-mediated immune response inhibition: an immune-escape mechanism



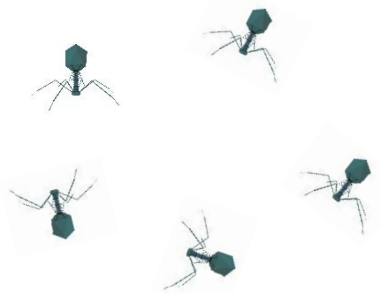
Up-regulation of IL-10



Viral infection and INFs inhibition: an immune-escape mechanism

IFN-alpha

IFN-beta



IFN-gamma

Virus inhibits innate immune response by IFNs down-regulation

NK activation

Tc activation

A novel, systemic, approach to *viral infections protection*

DIRECTIONS AND WAY OF ADMINISTRATION

- **Guna-Interferon gamma: 20 drops twice a day for 2 to 4 months (half dosage for children below 6 years)**
- **Guna-Interferon alpha: 20 drops twice a day for 2 to 4 months (half dosage for children below 6 years)**

Sublingual administration directly under the tongue or in a little water, preferibly far from meals.

INTERFERONE GAMMA E ALFA SONO PARTICOLARMENTE ATTIVI SULL'INNESCO DELLA RISPOSTA CITOLITICA

- **IFN-gamma** is able **to activate a cell-mediated immune response (IFN-gamma stimulates CD8 + to differentiate into cytotoxic T effector cells)** ideal against viruses. The Tc, in fact, operate the non-specific cytolysis of the cell infected with the virus (the Natural Killer - NK cells - instead, operate the specific cytolysis).

- **Interferon-alpha** (in some papers alpha seems to be favored over gamma; it is interesting how **Interferon-alpha prevents the virus from penetrating through the viropexy mechanism**, used by many viruses, into the cells not yet infected

*[**IFN-gamma** is also used by the body for the synthesis (conversion) into IFN-alpha (it is a bit like the mechanism of reciprocity between hormone T4 and T3, where T4 is the precursor of the hormone T3, true effector of the activity thyroid]*

guna.it

PRESCRIPTION ACCORDING TO THE AETIOLOGICAL DECISIONAL PROCESS

CYTOKINE	STRENGTHENING same cytokine	MODULATION opposing cytokine
IL-4	IL-4 4C	INF-gamma 4C/IL-12 4C
IL-5	IL-5 4C	TGF-beta 4C
IL-6	IL-6 4C	IL-10 4C
IL-7	IL-7 4C	IL-10 4C/TGF-β1 4C
IL-8	IL-8 4C	IL-10 4C/TGF-β1 4C
IL-9	IL-9 4C	IL-10 4C
IL-10	IL-10 4C	IL-1 4C/TNF 4C/IL-6 4C
IL-11	IL-11 4C	IL-2 4C
IL-12	IL-12 4C	IL-4 4C/IL-10 4C
TGF-beta 1	TGF-beta 4C	IL-12 4C
TNF	TNF-alpha 4C	Guna Anti IL-1 4C+IL-10 4C

…and we need to counteract the ovrexpression of IL-10

CITOMIX

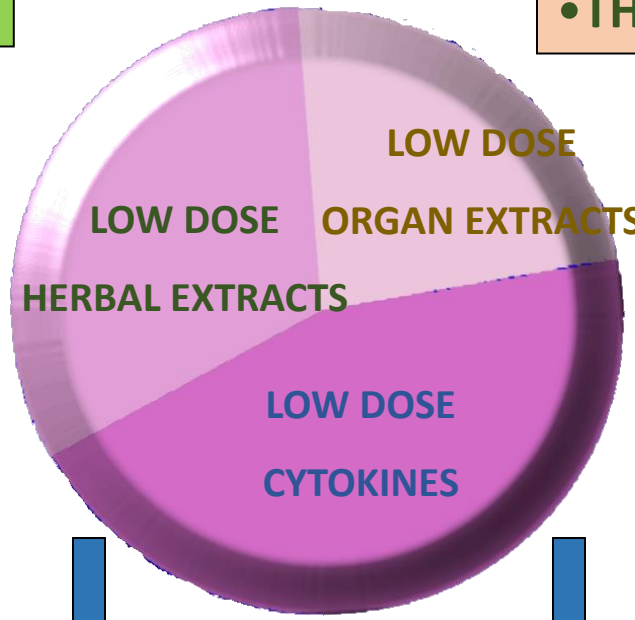
- VACCINIUM VITIS
- ANANASSA SATIVA
- HYDROCOTYLE ASIATICA (CENTELLA ASIATICA)

ANTINFLAMMATORY AND ANTIOXIDANT ACTION (*Vaccinium vitis*),

RES STIMULATION (*Ananassa sativa*),

ANTINFIAMMATORY ACTION (*Hydrocotyle asiatica*)

- VASA LYMPHATICA SUIS
- MEDULLA OSSIS SUIS
- THYMULINE



TARGETED ANTINFLAMMATORY ACTION; STIMULATION OF IMMUNOCOMPETENT TISSUES

- IL-4
- IL-2

B PROLIFERATION AND APC BOOSTING (IL-4); B, T AND NK STIMULATION (IL-2)

- GCSF
- IL1-beta
- INF -gamma
- IL-6

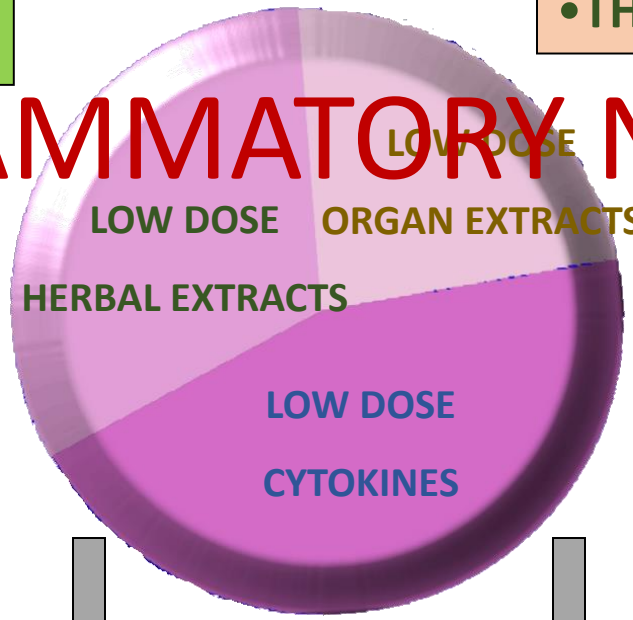
ONSET OF THE IMMUNE RESPONSE

CITOMIX

- VACCINIUM VITIS (MOUNTAIN CRANBERRY)
- ANANASSA SATIVA
- HYDROCOTYLE ASIATICA (CENTELLA ASIATICA)

- VASA LYMPHATICA SUIS
- MEDULLA OSSIS SUIS
- THYMULINE

ANTINFLAMMATORY NETWORK



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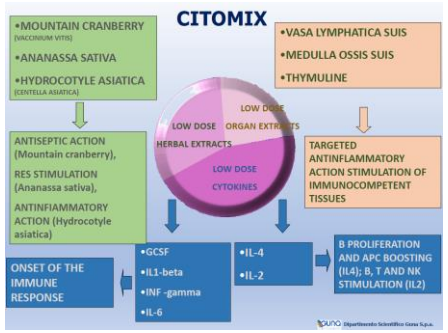
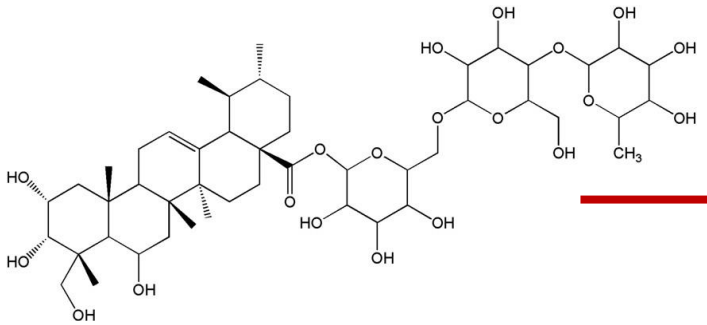


Hydrocotyle asiatica L. 3X
Centella asiatica

EFFECTS
- *Anti-oxidant*
- *Anti-inflammatory*

MADECASSOSIDE



- Nitric Oxide (NO)
- Prostaglandin E2 (PGE2)
- TNF-α
- Interleukin-1β (IL-1β)
- IL-6

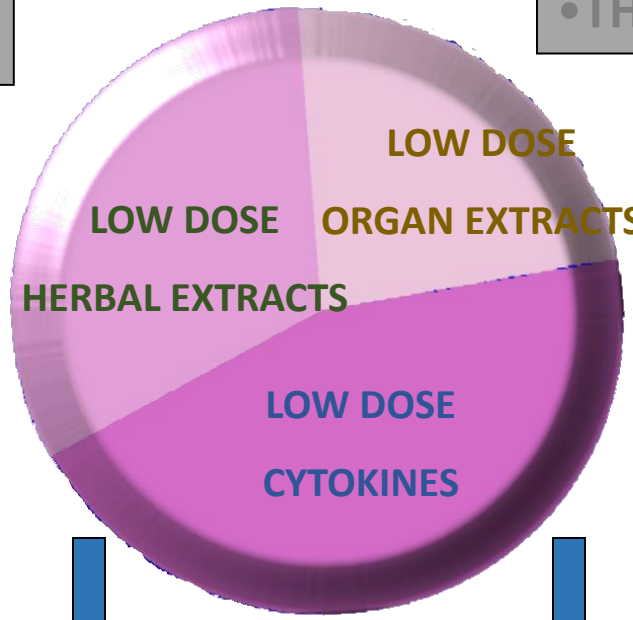
Madecassoside (MA), major triterpenoid component of *Centella asiatica*

Cao W, Li XQ, Zhang XN, Hou Y, Zeng AG, Xie YH, Wang SW. Madecassoside suppresses LPS-induced TNF-alpha production in cardiomyocytes through inhibition of ERK, p38, and NF-kappaB activity. Int Immunopharmacol. 2010;10(7):723-9.

CITOMIX

- VACCINIUM VITIS (MOUNTAIN CRANBERRY)
- ANANASSA SATIVA
- HYDROCOTYLE ASIATICA (CENTELLA ASIATICA)

- VASA LYMPHATICA SUIS
- MEDULLA OSSIS SUIS
- THYMULINE



ANTINFLAMMATORY AND ANTIOXIDANT ACTION (*Vaccinium vitis*),

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TARGETED ANTINFLAMMATORY ACTION; STIMULATION OF IMMUNOCOMPETENT TISSUES

ONSET OF THE IMMUNE RESPONSE

- GCSF
- IL1-beta
- INF -gamma
- IL-6

- IL-4
- IL-2

B PROLIFERATION AND ADJUVANTING (IL-4); B, T AND NK STIMULATION (IL-2)

CITOMIX – COMPONENTS DESCRIPTION -2

BIOLOGICAL ORIGIN COMPONENTS

G-CSF

G-CSF is a pleiotropic hemopoietic growth factor that regulates the proliferation and differentiation of progenitor cells within the bone marrow and the release of mature neutrophils into the peripheral blood increasing ADCC (Antibody Dependent Cell-Mediated). and superoxide anion production.

IL-1β

IL-1β is normally produced in response to infection, injury, or immunologic challenge modulating the proinflammatory response triggering innate immunity and activating B and T lymphocytes.

IL-2

IL-2 is a growth factor for T-cells and B-lymphocytes. In addition, it stimulates antibodies production and secretion; IL-2 participate with IL-12 to increase NK cytotoxicity.

IL-4

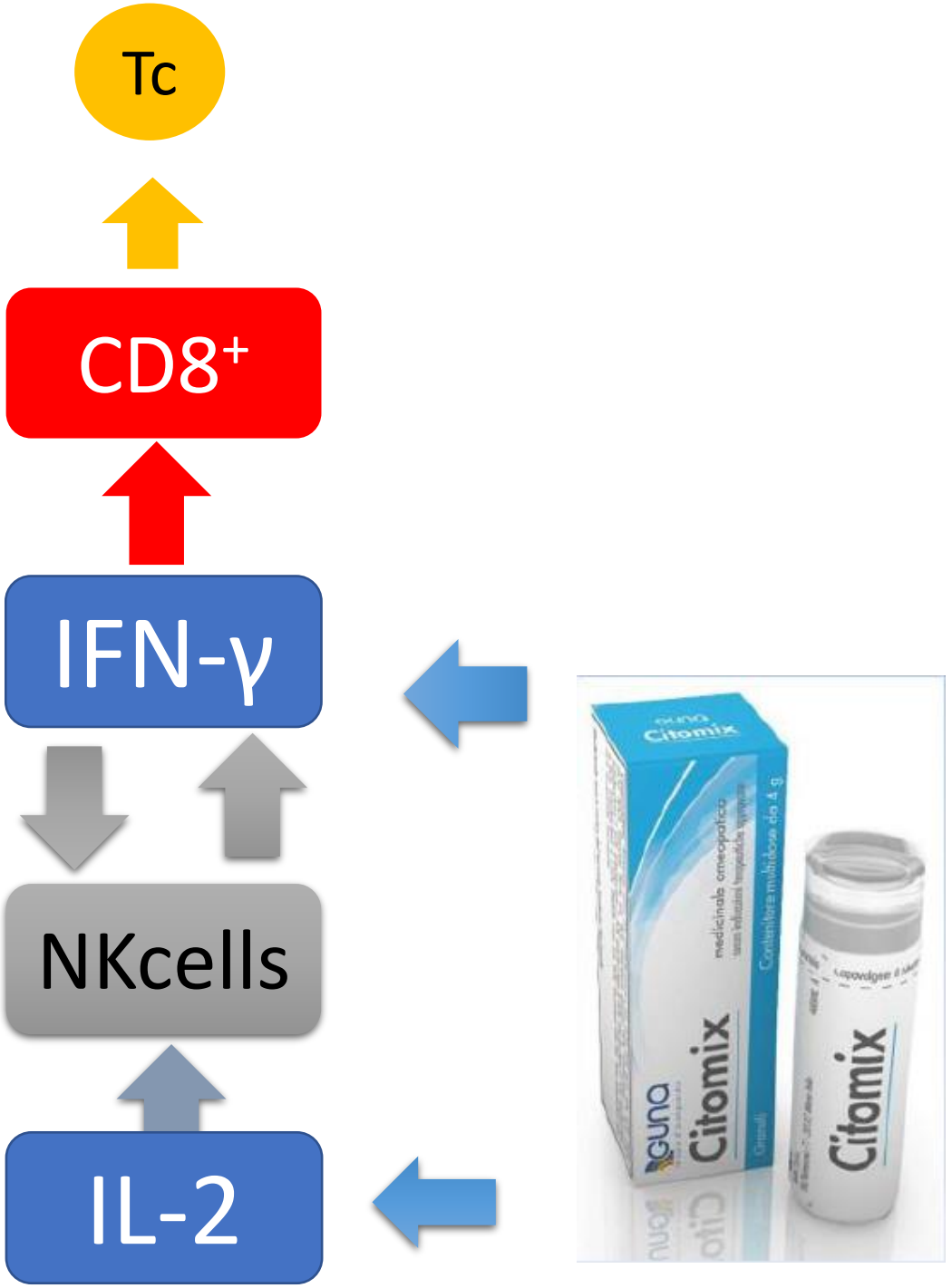
IL-4 is a cytokine that participates in the regulation of the immune system at multiple levels, in particular, IL-4 drives the expansion of B-lymphocytes clones and activates the alternative macrophage response.

IFN-γ

Interferon-γ (IFN-γ) exerts a strong immunoregulatory action on several cells driving the Th0 lymphocytes differentiation to Th1 inducing the maturation of $CD8^+$, macrophages activation and NK cytotoxicity. It is crucial for immunity against intracellular pathogens and exerts potent phagocyte-activating effect.

IL-6

IL-6 is a multifunctional cytokine involved both in inflammation and infection responses inducing the synthesis of acute phase proteins. It also in the regulation of metabolic and regenerative processes.


Tc
CD8+
IFN-γ
NKcells
IL-2

guna
Citomix
medicinale omeopatico
Citomix

Immunology Letters 203 (2018) 95–101

Contents lists available at ScienceDirect

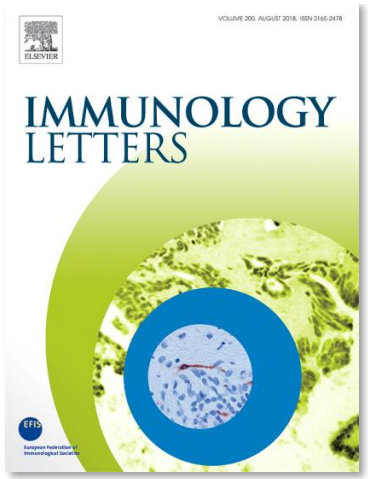
Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Low-dose multicomponent medication modulates humoral and cellular immune response in an *ex-vivo* study on children subjected to adenoid surgery

Sara Carlotta Tagliacarne[a], Chiara Valsecchi[b], Marco Benazzo[c], Michele Nichelatti[d], Alessia Marseglia[b], Giorgio Ciprandi[e,*], Sergio Bernasconi[f]

[a] *Department of Clinical Surgical Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy*
[b] *Department of Pediatrics, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy*
[c] *Department of Otolaryngology, University of Pavia and Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy*
[d] *Service of Biostatistics Hematology Department, Niguarda Ca' Granda Hospital, Milan, Italy*
[e] *Department of Medicine, IRCCS-Ospedale Policlinico San Martino, Genoa, Italy*
[f] *Department of Pediatrics, University of Parma, Parma, Italy*



R.R.I. (Recurrent Respiratoty Infections) - EPIDEMIOLOGY

EPIDEMIOLOGY

- Under 5 years of age: **20%** of population suffer for RRI
- **1/3** of pediatric consultations and **8-18%** of hospitalizations
- **Il 25-45%** of children with RRI needs **surgery (adeno-tonsillar Hypertrophy)**

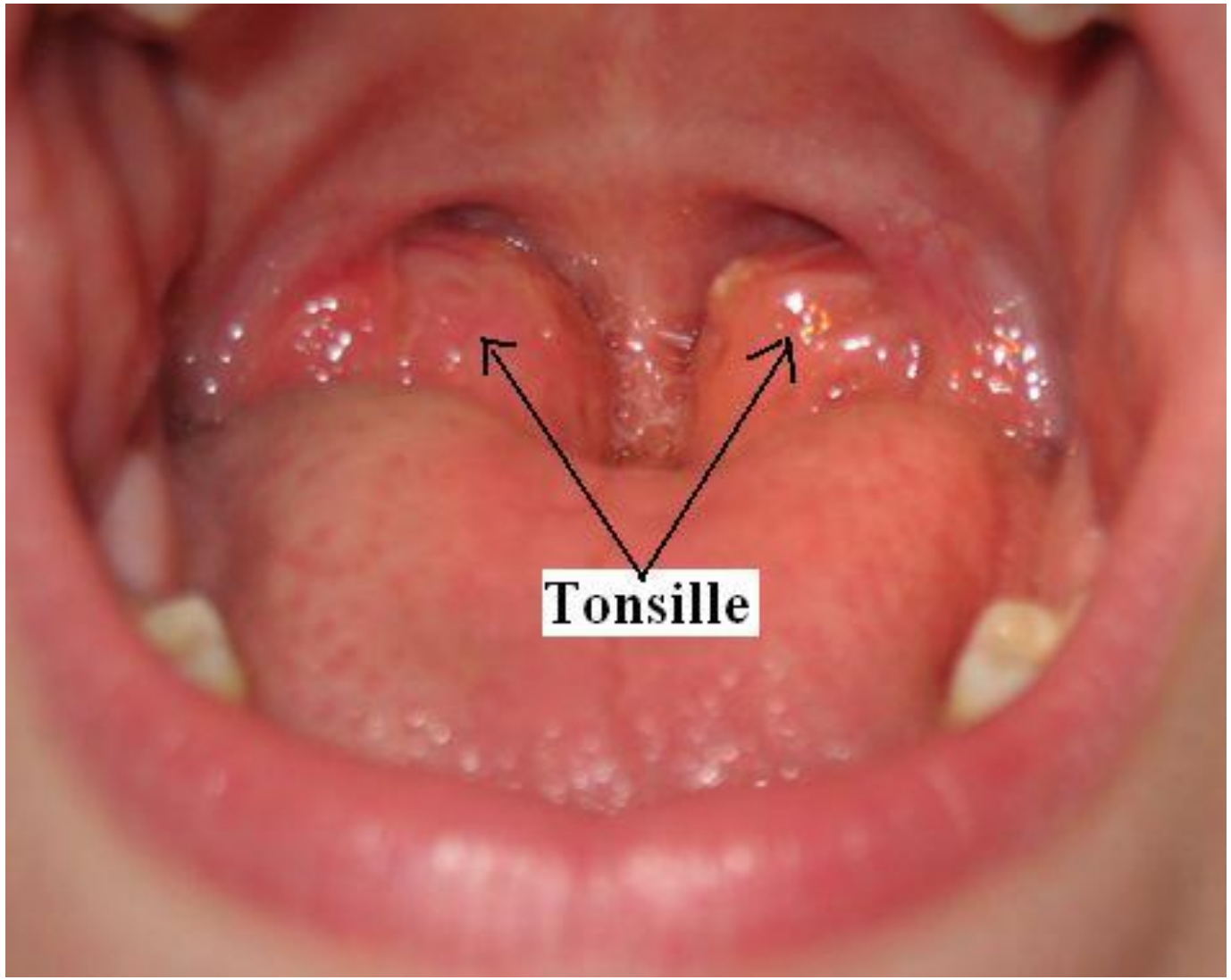
DIAGNOSIS:

- **More than 6 infections/year**
- **More than 1 infection/month between September and April**

TREATMENTS

Mainly symptomatic **(corticosteroids and acetominophen)** and prophylactic **(antibiotics).**

Bacterial lysates



Tortora, Derrickson *Conosciamo il corpo umano* © Zanichelli editore 2009

STUDIO CITOMIX - SCOPO

Valutare l'attività immunomodulante "in vitro" di CITOMIX

- AMCs (*Adenoidal Mononuclear Cells*) di pazienti pediatrici.

Parametri immunologici:

- capacità proliferativa delle sottopopolazioni linfocitarie B
- produzione di citochine e immunoglobuline.

CRITERI DI INCLUSIONE:

- necessità di asportazione chirurgica delle adenoidi.

CRITERI DI ESCLUSIONE:

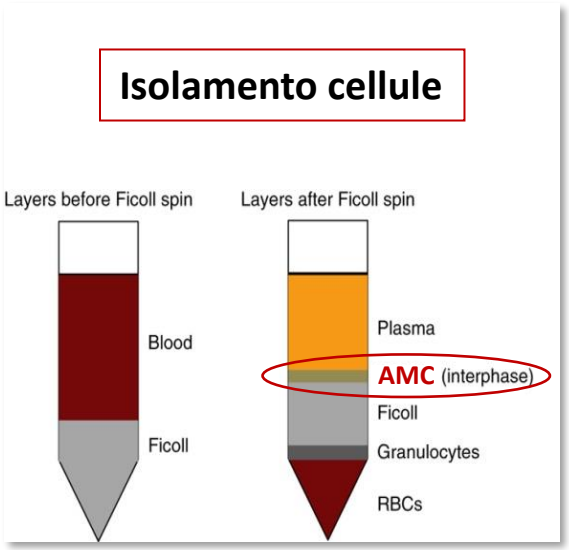
- condizioni di immunodeficienza,
- presenza di patologie autoimmune,
- obesità,
- assunzione cronica di farmaci steroidei,
- presenza di disabilità fisiche e psichiche.

ATTIVITA' IMMUNOMODULANTE DI CITOMIX -*DISEGNO DELLO STUDIO*

Campione: adenoidi di **50 soggetti pediatrici** (35 maschi, 15 femmine, età media 6 anni) con Infezioni Respiratorie Ricorrenti (IRR)

Schiacciamento meccanico

Isolamento cellule



Espansione delle AMC *in vitro*

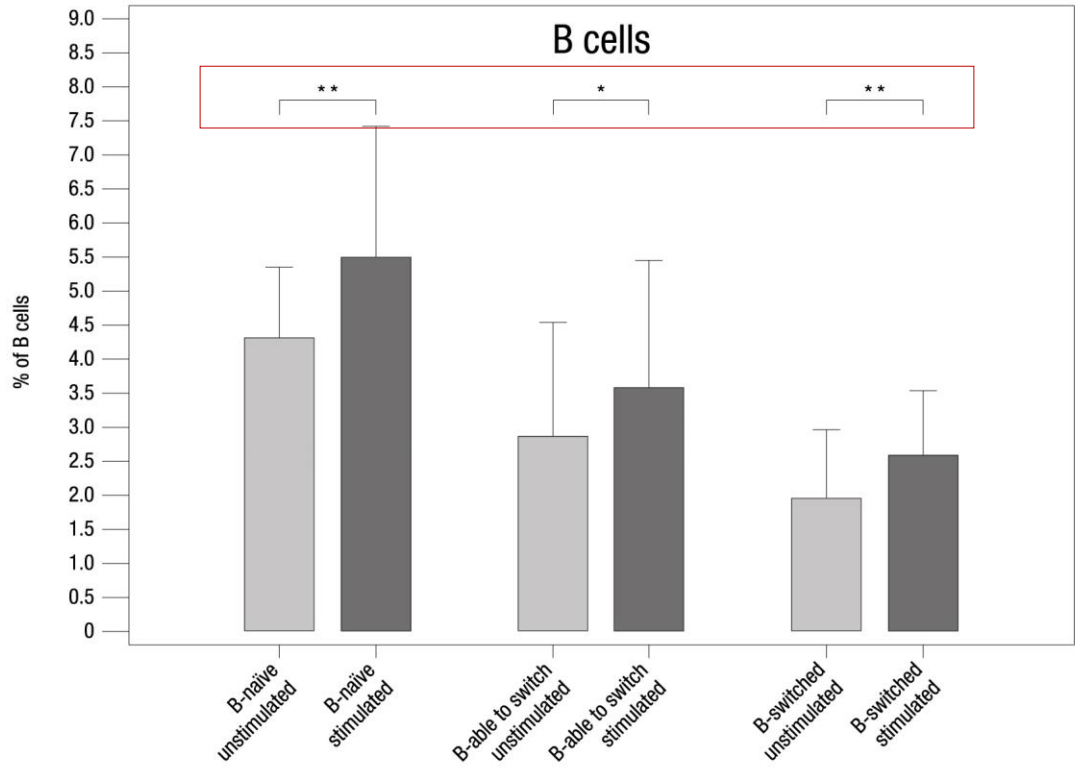
Quantificazione delle citochine
- **IL-6**
- **IL-10**
- **IFN-γ**

Quantificazione delle immunoglobuline
- **IgA**
- **IgG**
- **IgM**

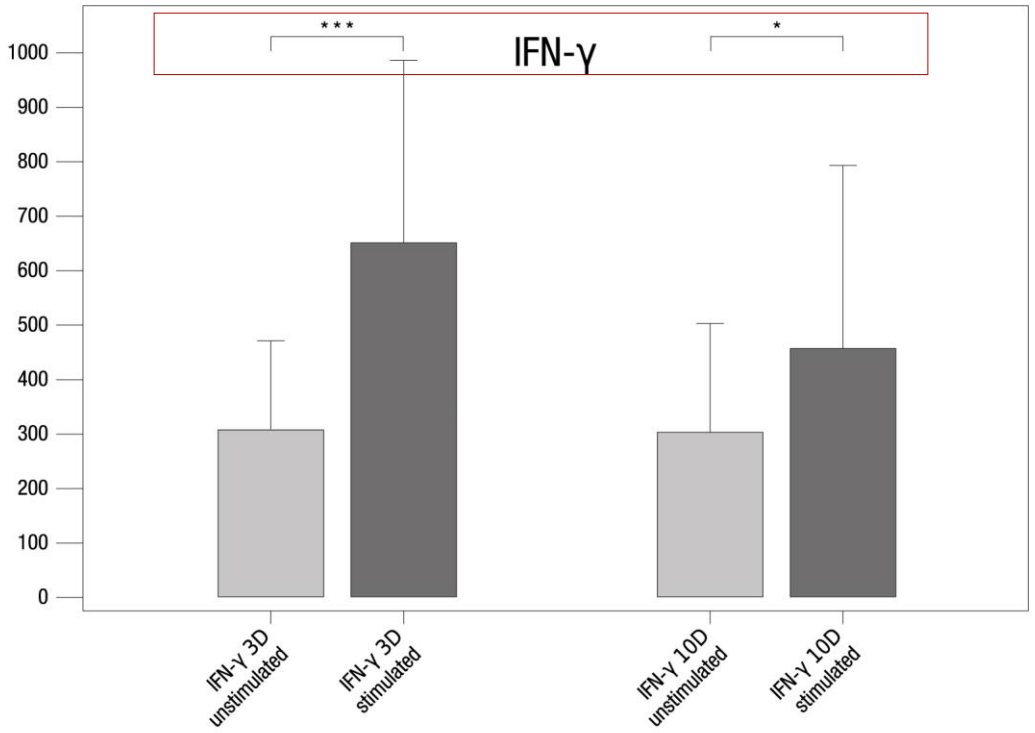
Caratterizzazione dei sub-cloni di Linfociti B

RESULTS

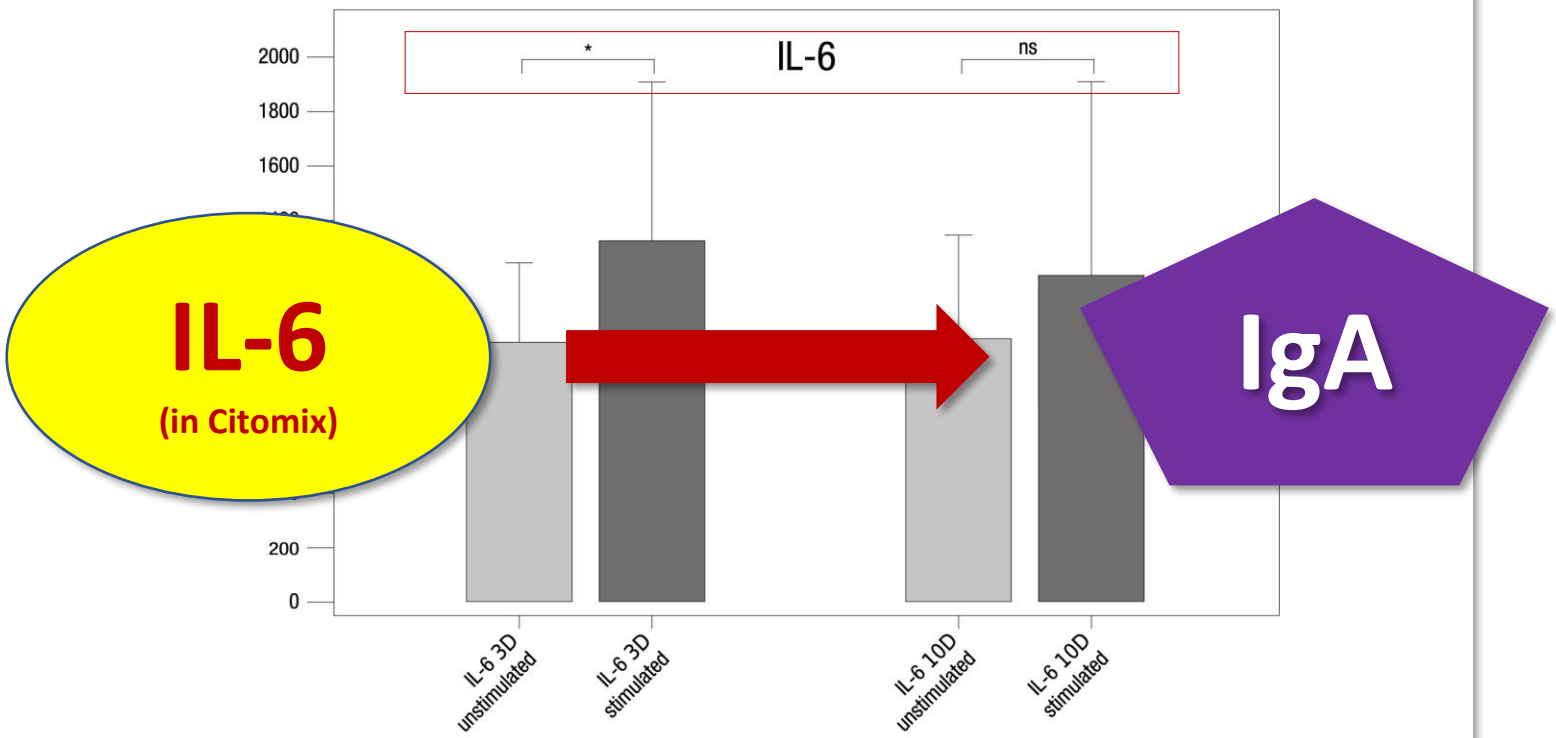
CITOMIX INDUCES A SIGNIFICANT INCREASE OF B *NAÏVE*, B *ABLE TO SWITCH* AND B *SWITCHED*



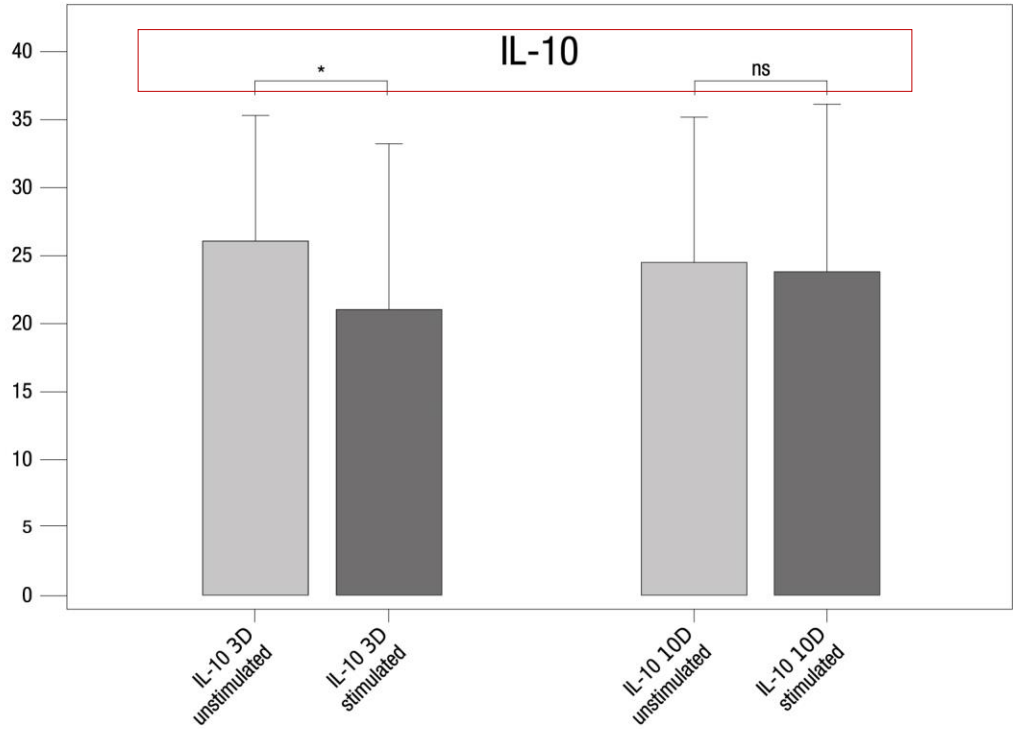
CITOMIX INDUCES A SIGNIFICANT INCREASE OF IFN-γ AFTER 3 AND 10 TREATMENT DAYS



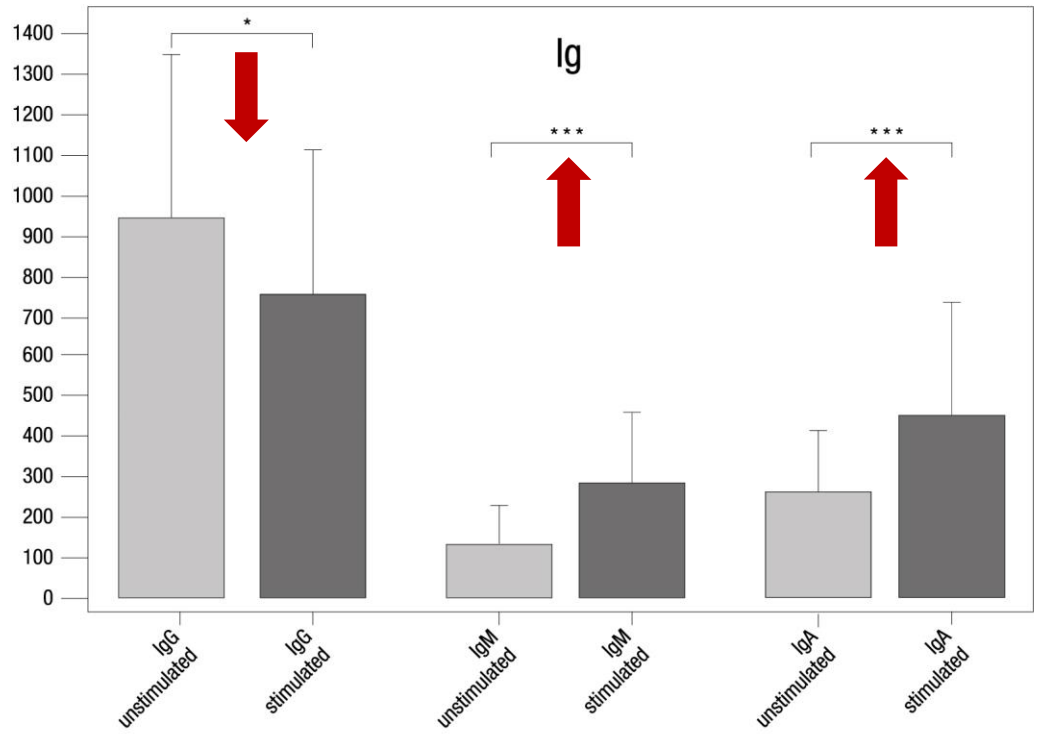
CITOMIX INDUCES A SIGNIFICANT INCREASE OF IL-6 AFTER 3 AND 10 TREATMENT DAYS



CITOMIX INDUCES A SIGNIFICANT DECREASE OF IL-10 AFTER 3 AND 10 TREATMENT DAYS



CITOMIX INDUCES A SIGNIFICANT INCREASE OF IgA AND IgM AFTER 3 AND 10 TREATMENT DAYS

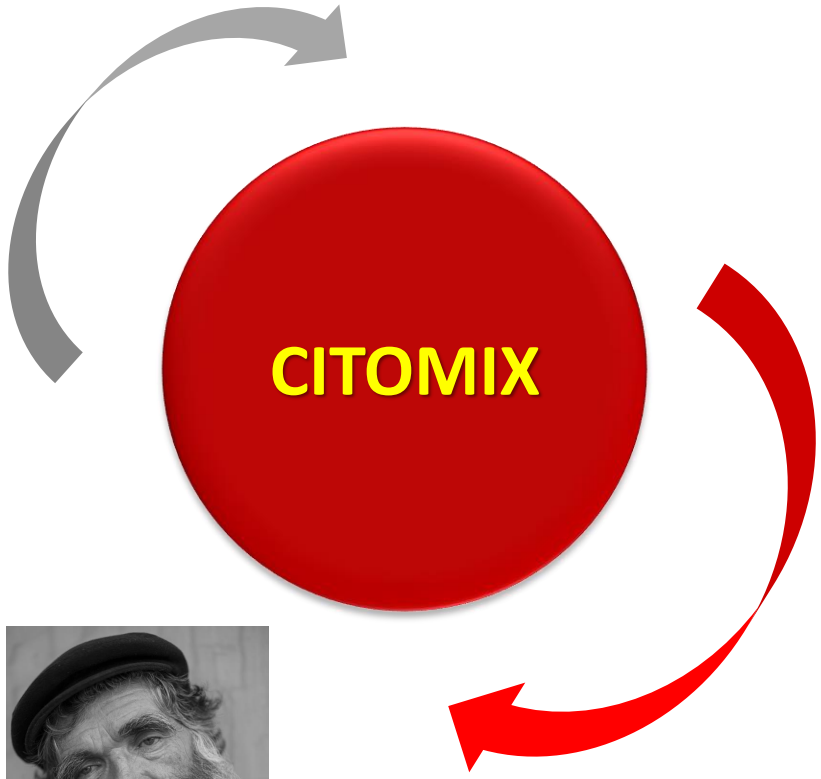


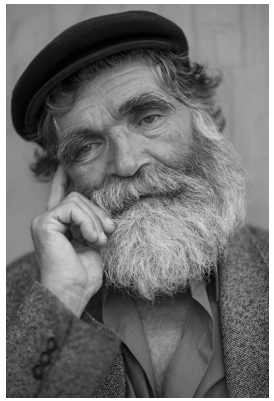
DIRECTIONS

- **<u>Prevention:</u>** 5 granules twice a day, every day, for 3 month.
  Children under 6 years: 5 pellets once a day, for 3 months

In conclusion, TO SUMMARIZE

GUNA-Interferon-gamma

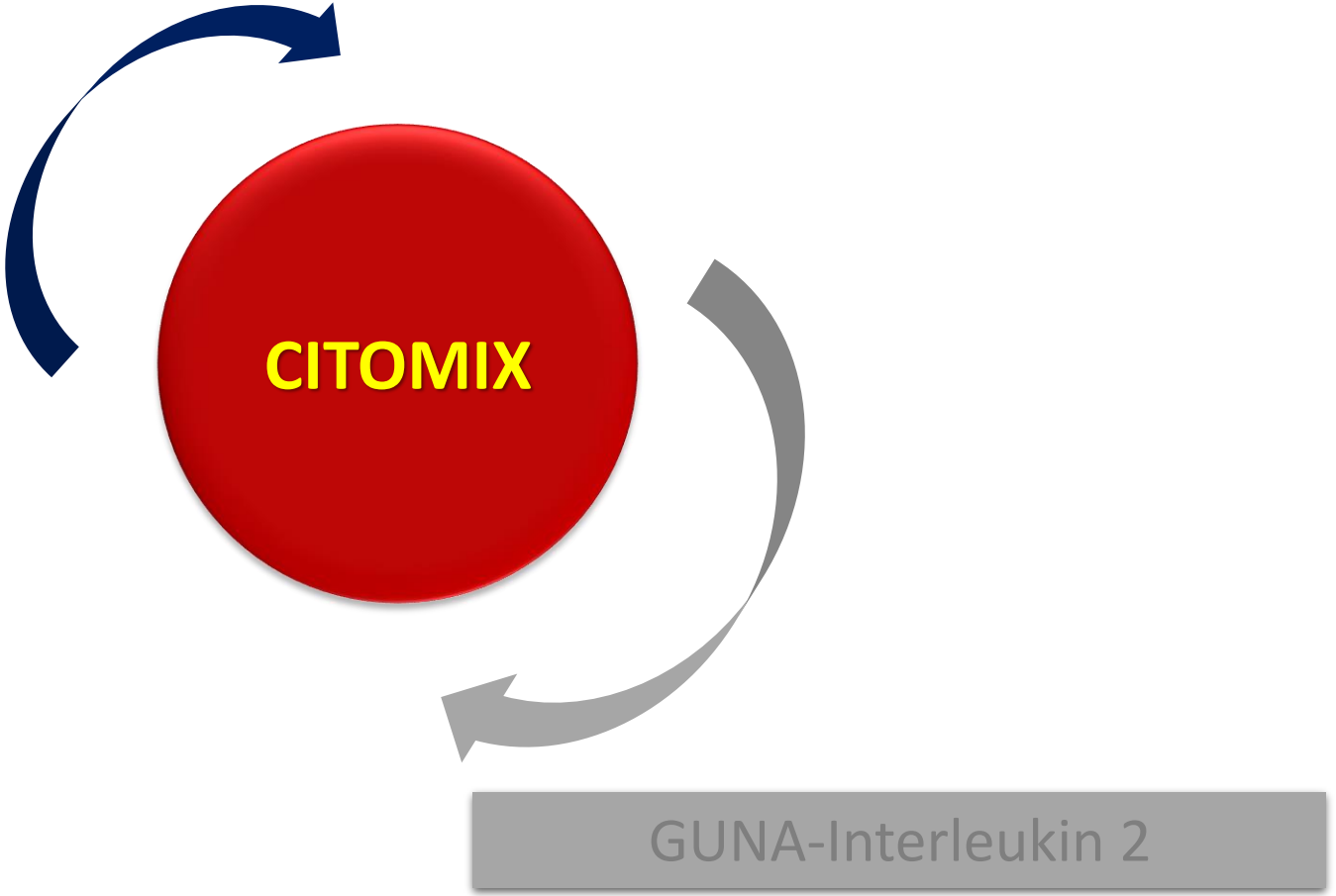




GUNA-Interleukin 2

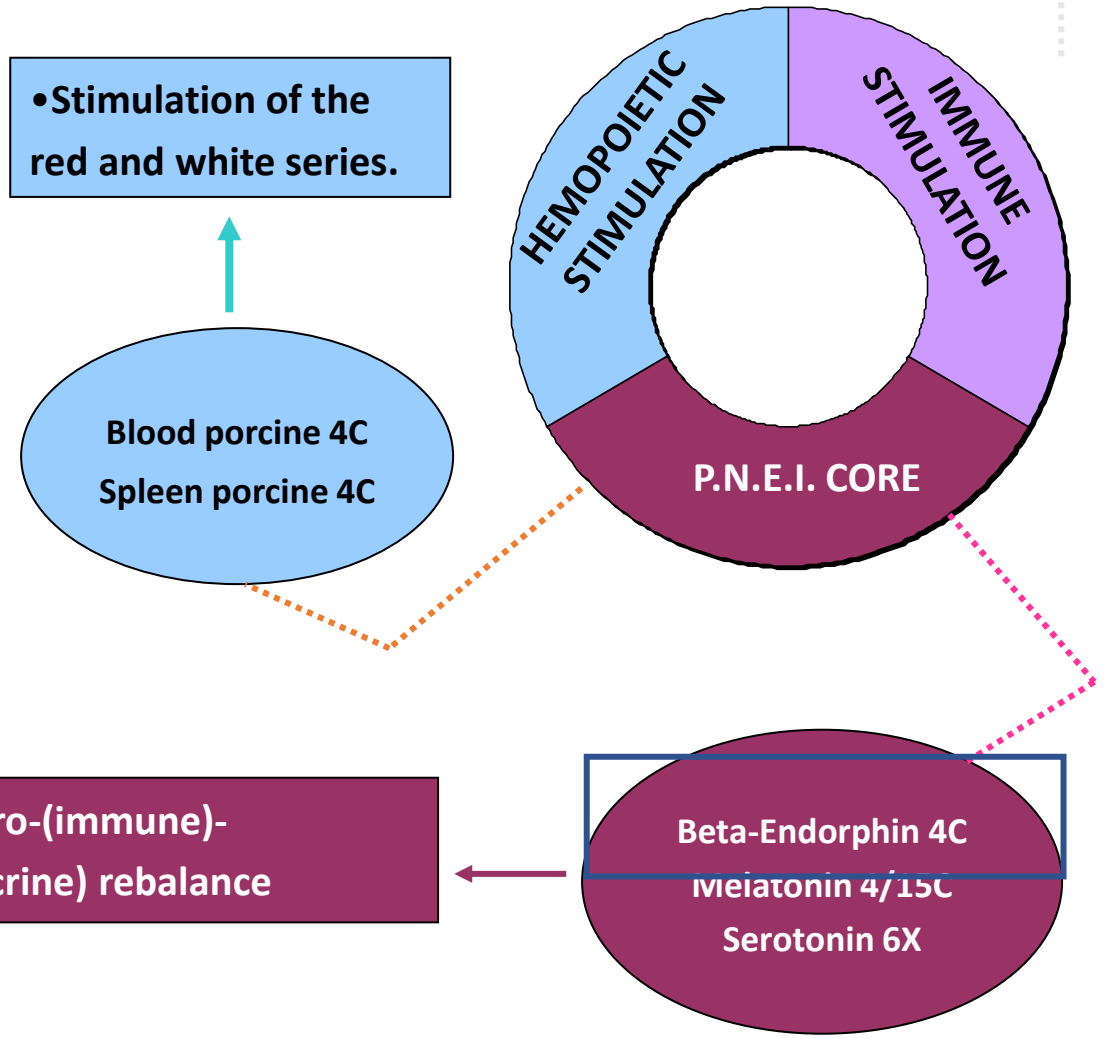
In conclusion, TO SUMMARIZE

GUNA-Interferon-gamma



GUNA-Interleukin 2

GUNA-VIRUS



β-ENDORPHIN and Immune System modulation

Enhances

- IL-4
- Antigen Presenting Cells
- antibody titer
- NK cells

β-ENDORPHIN
(in presence of a mitogen agent e.g. LPS)

Th2 (humoral response)

Relaxing techniques induce β-endorphin production and
Improve melanoma and breast cancer surviving rate
enhancing NK cell activity and reducing inflammation

!

Sauriyal DS, Jaggi AS, Singh N. Extending pharmacological spectrum of opioids beyond analgesia: multifunctional aspects in different pathophysiological states. Neuropeptides. 2011 Jun;45(3):175-88.

Gein SV, Baeva TA, Nebogatikov VO, Tendryakova SP. β-Endorphin effects on antibody production, proliferation, and secretion of Th1/Th2 cytokines in vivo. Bull Exp Biol Med. 2012 Mar;152(5):595-9.

Mahbub-E-Sobhani, Haque N, Salma U, Ahmed A. Immune modulation in response to stress and relaxation. Pak J Biol Sci. 2011 Mar 15;14(6):363-74.

Turkey tail - Coriolus versicolor

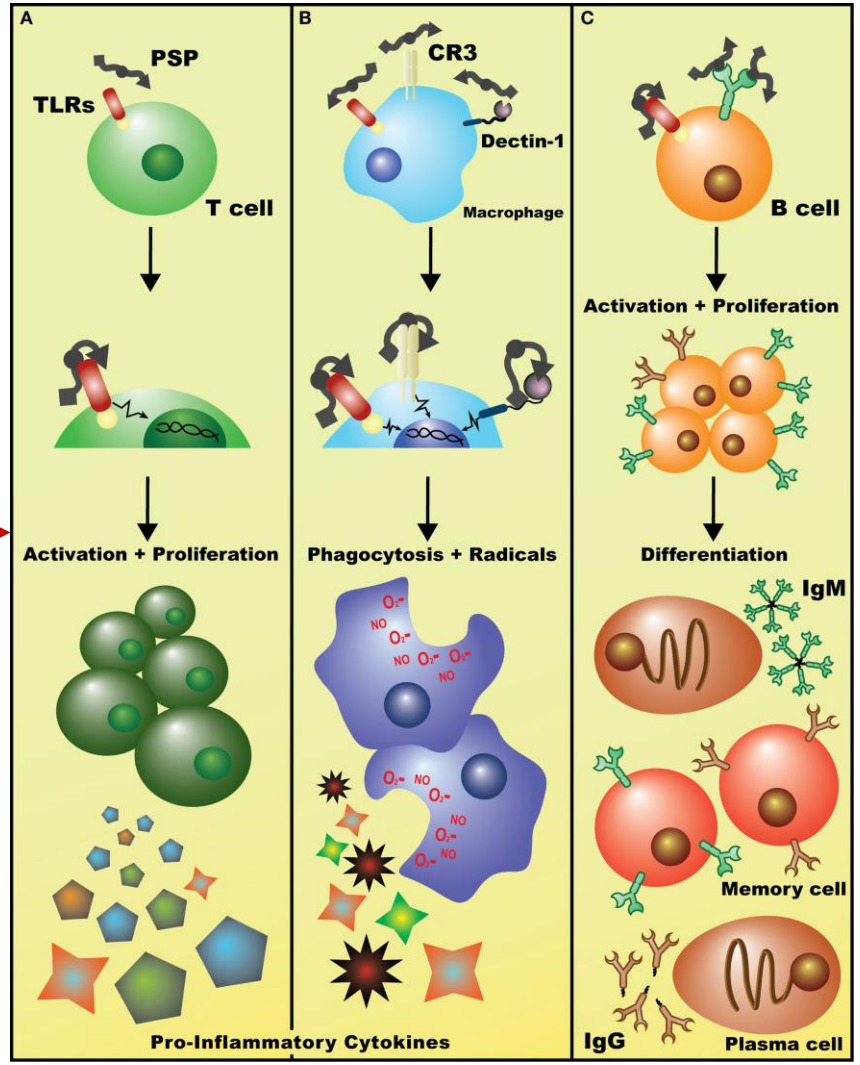
Polysaccharopeptide (PSP)
Immunomodulating properties
with pro-inflammatory tendency

PBMCs activation *via* TLRs → TNF-α, IL-1β ↑

Splenic lymphocytes → IFN-γ, IL-2 ↑

TGF-β

IL-6 → Enhancement of B cell antibody production and B helper activity, inhibition of Treg differentiation



- *Saleh MH, Rashedi I, Keating A. Immunomodulatory Properties of Coriolus versicolor: The Role of Polysaccharopeptide. Front Immunol. 2017;8:1087. doi:10.3389/fimmu.2017.01087*
- *Yang SF, Zhuang TF, Si YM, Qi KY, Zhao J. Coriolus versicolor mushroom polysaccharides exert immunoregulatory effects on mouse B cells via membrane Ig and TLR-4 to activate the MAPK and NF-κB signaling pathways. Mol Immunol. 2015;64(1):144-151. doi:10.1016/j.molimm.2014.11.007*

Ling chin mushroom - Ganoderma lucidum

G. lucidum active compounds

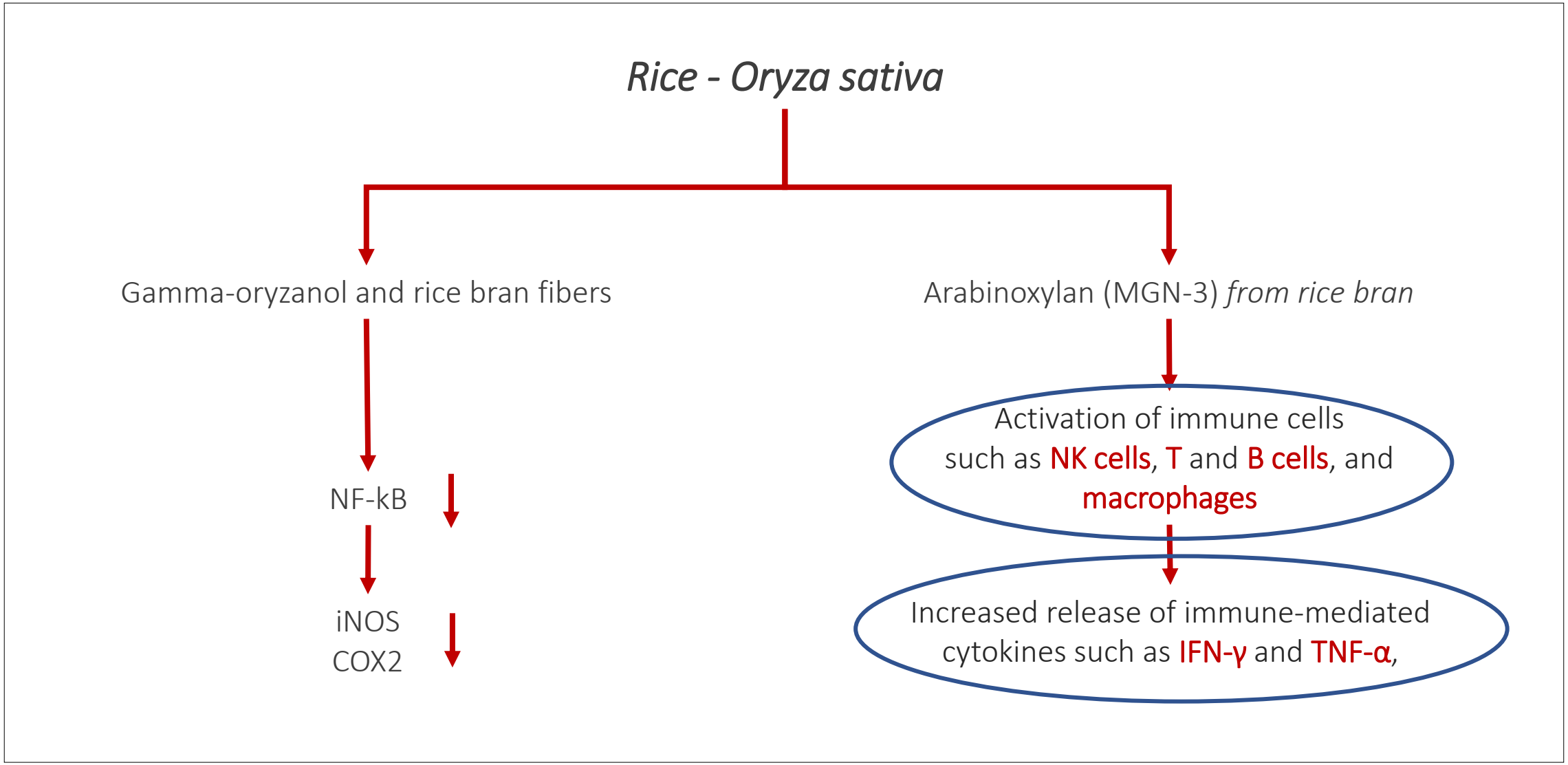
Polysaccharides

Biological activity	Type of compound
1. Antitumor activities	Polysaccharides and triterpenoids
2. Hypoglycemic activity	Polysaccharides
3. Immunomodulation and stimulation of cytokine production	Polysaccharides
4. Immunomodulation	Protein (LZ-8)
5. Cytotoxicity to hepatoma cells	Triterpenoids
6. Hepato-protection	Triterpenoids
7. Inhibition of histamine release	Triterpenoids
8. Inhibition of cholesterol synthesis	Triterpenoids
9. Inhibition of protein farnesylation (Ras)	Triterpenoids
10. stimulation of platelet aggregation	Triterpenoids
11. Inhibition of platelet aggregation	Adenosine/triterpenoids
12. Anti-HIV/antiviral activity	Triterpenoids
13. Induction of apoptosis	Triterpenoids

PBMCs activation *via* TLR-4

IL-1β

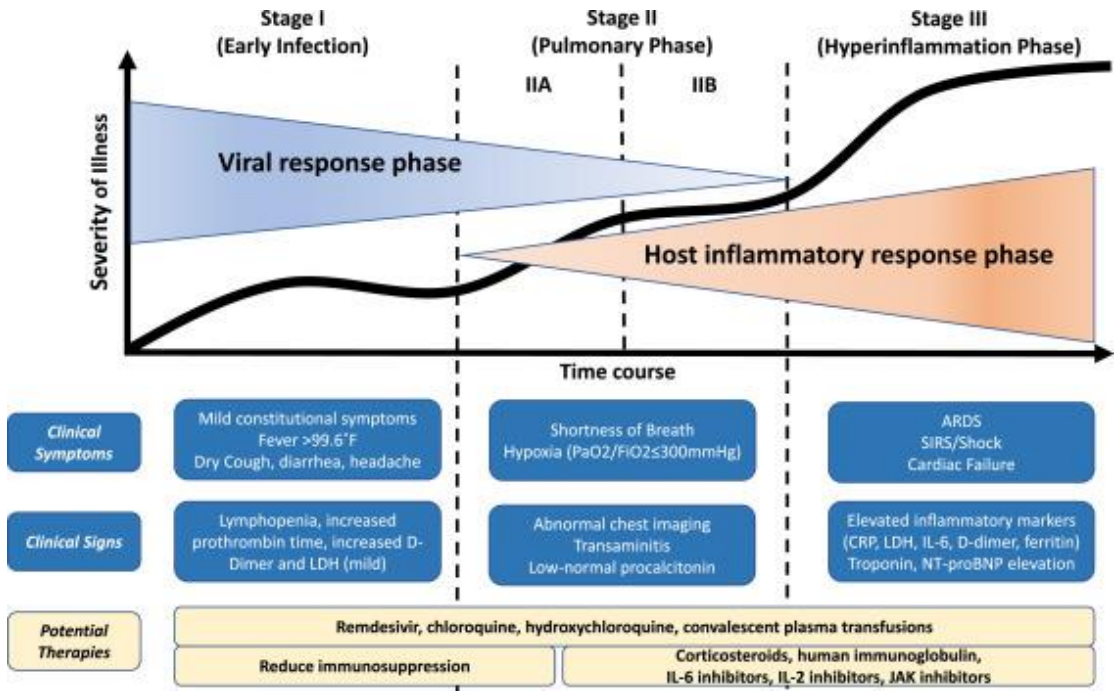
- *Shao BM, Dai H, Xu W, Lin ZB, Gao XM. Immune receptors for polysaccharides from Ganoderma lucidum. Biochem Biophys Res Commun. 2004;323(1):133-141. doi:10.1016/j.bbrc.2004.08.069*
- *Shiao MS. Natural products of the medicinal fungus Ganoderma lucidum: occurrence, biological activities, and pharmacological functions. Chem Rec. 2003;3(3):172-180. doi:10.1002/tcr.10058*
- *Xu Z, Chen X, Zhong Z, Chen L, Wang Y. Ganoderma lucidum polysaccharides: immunomodulation and potential anti-tumor activities. Am J Chin Med. 2011;39(1):15-27. doi:10.1142/S0192415X11008610*



- *Liao HF, Chen YY, Yang YC, Wang CS, Chen YJ. Rice (Oryza sativa L.) inhibits growth and induces differentiation of human leukemic U937 cells through activation of peripheral blood mononuclear cells. Food Chem Toxicol. 2006;44(10):1724-1729. doi:10.1016/j.fct.2006.05.015*
- *Park HY, Lee KW, Choi HD. Rice bran constituents: immunomodulatory and therapeutic activities. Food Funct. 2017;8(3):935-943. doi:10.1039/c6fo01763k*
- *Toda M. Rice Components with Immunomodulatory Function. J Nutr Sci Vitaminol (Tokyo). 2019;65(Supplement):S9-S12. doi:10.3177/jnsv.65.S9*

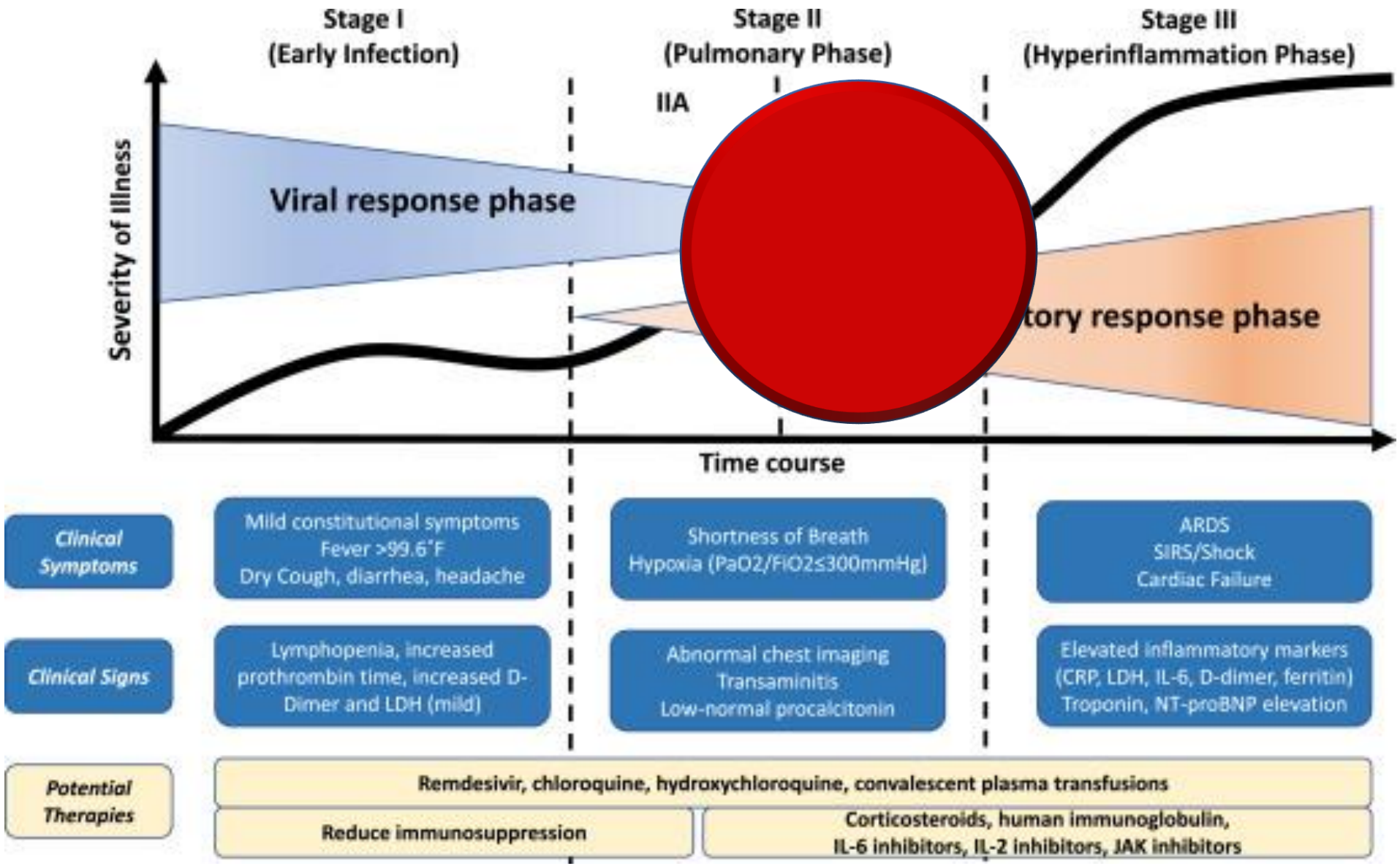
Our (unique) goal

- *Before*
- ***During***
- ***After***



And what to do during an infective disease?

(in overlapping with other treatments)



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Keep in mind these drugs

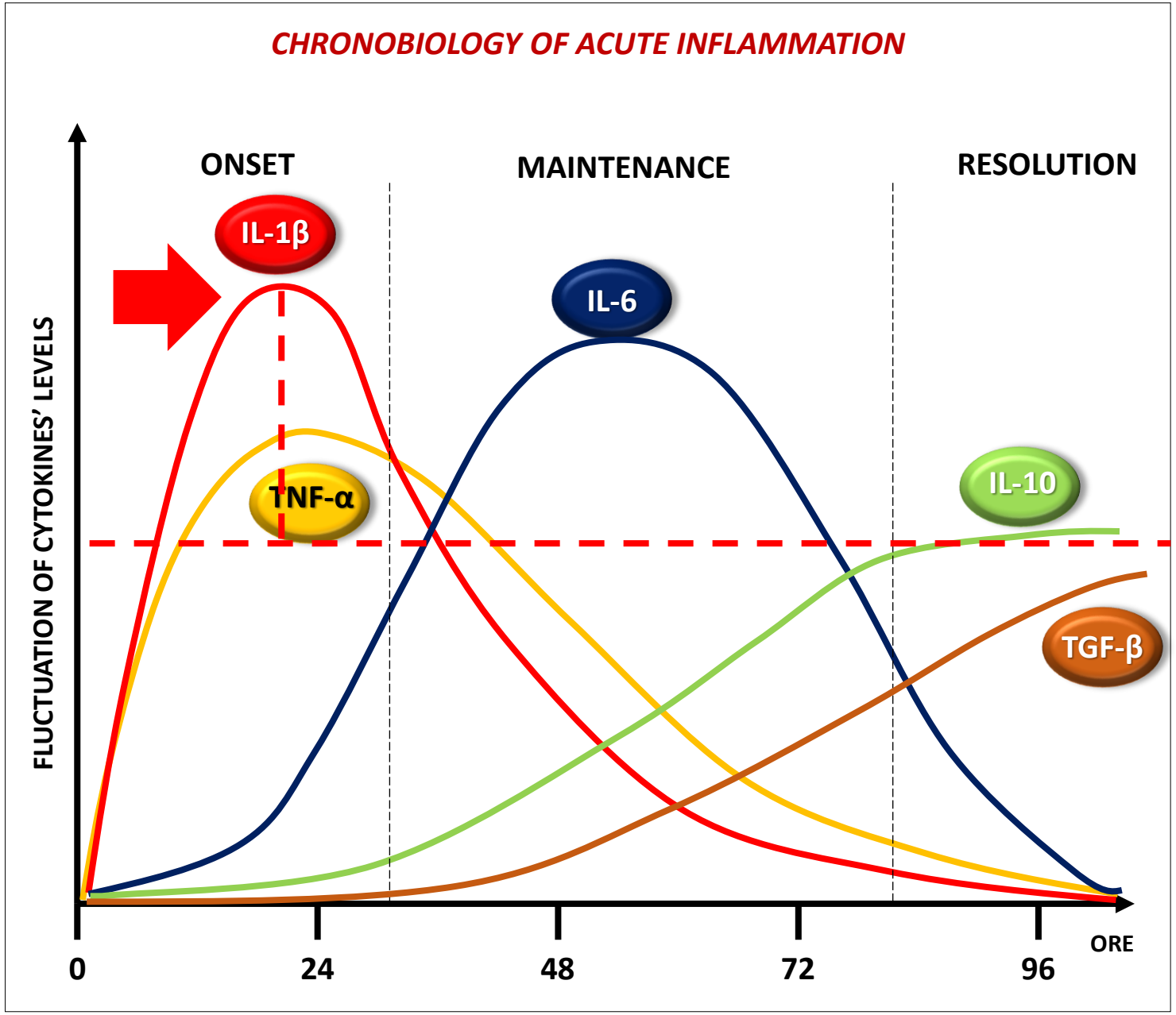
Emapalumab	IFN-α
Anakinra	IL-1RA
Sarilumab	IL-6RA
Tocilizumab	IL-6

Covid-19, AIFA autorizza tre nuovi studi per sperimentazioni di farmaci per il trattamento dell'infezione da nuovo coronavirus

L'Agenzia Italiana del Farmaco ha dato il via libera a nuovi studi per la sperimentazione clinica di tre medicinali: **Emapalumab e Anakinra, Sarilumab, e Tocilizumab** per trattare la malattia Covid-19,determinata dall'infezione da nuovo coronavirus.

Study Type :	Interventional (Clinical Trial)
Estimated Enrollment :	342 participants
Allocation:	Randomized
Intervention Model:	Factorial Assignment
Masking:	None (Open Label)
Primary Purpose:	Treatment
Official Title:	A Prospective, Randomized, Factorial Design, Interventional Study to Compare the Safety and Efficacy of Combinations of Blockade of Interleukin-6 Pathway and Interleukin-1 Pathway to Best Standard of Care in Improving Oxygenation and Short- and Long-term Outcome of COVID-19 Patients With Acute Hypoxic Respiratory Failure and Systemic Cytokine Release Syndrome
Estimated Study Start Date :	April 2020
Estimated Primary Completion Date :	September 2020
Estimated Study Completion Date :	December 2020

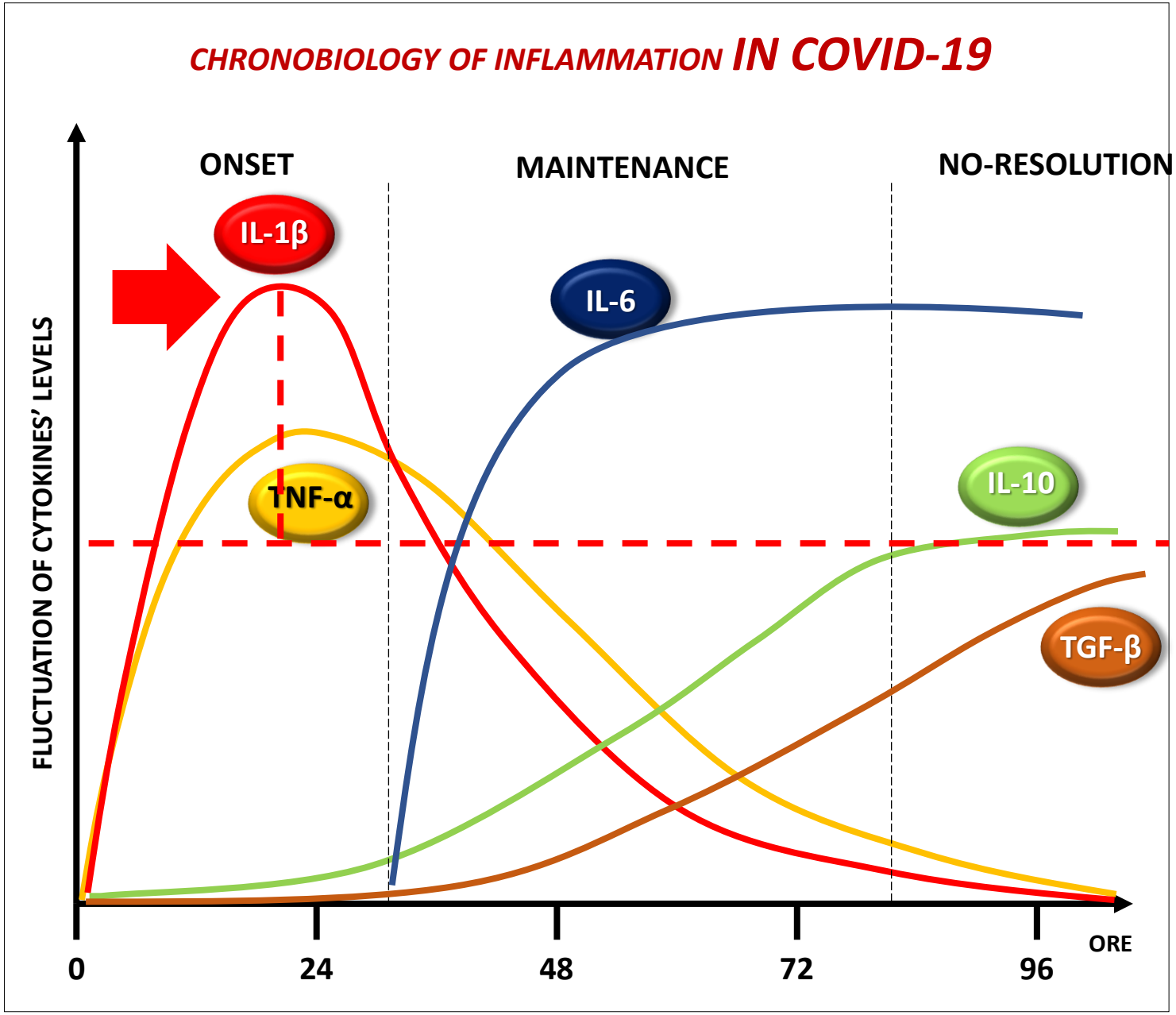
Condition or disease	Intervention/treatment	Phase
COVID-19	Other: Usual Care Drug: **Anakinra Drug**: Siltuximab Drug: Tocilizumab	Phase 3



Petersen AM[1], Pedersen BK. The anti-Inflammatory effect of exercise. J Appl Physiol (1985). 2005 Apr;98(4):1154-62

Modificata a fini didattici.

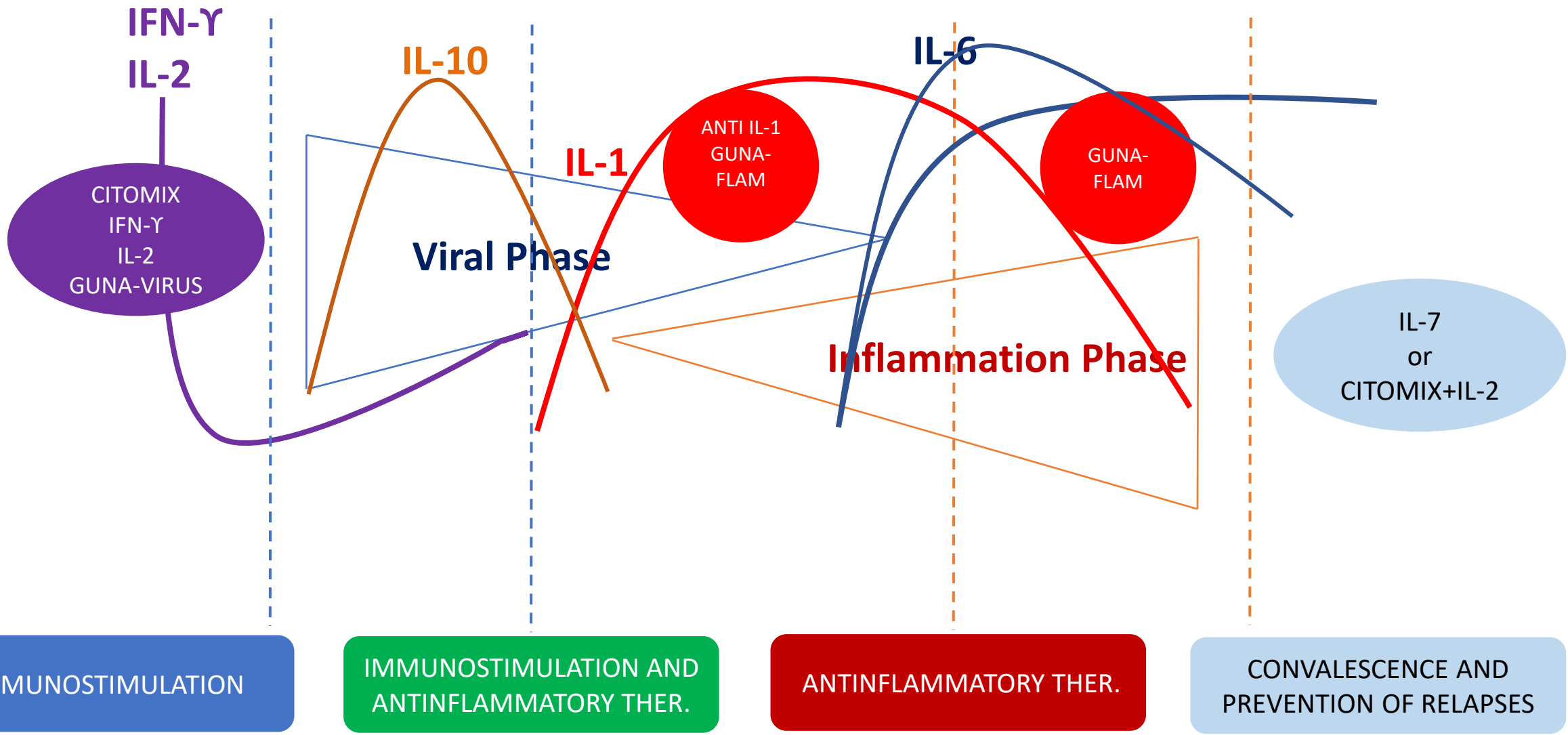
CHRONOBIOLOGY OF INFLAMMATION IN COVID-19



Petersen AM[1], Pedersen BK. The anti-Inflammatory effect of exercise. J Appl Physiol (1985). 2005 Apr;98(4):1154-62

Modificata a fini didattici.

Space-Time Immunomodulation



IMMUNOSTIMULATION

IMMUNOSTIMULATION AND ANTINFLAMMATORY THER.

ANTINFLAMMATORY THER.

CONVALESCENCE AND PREVENTION OF RELAPSES

Space-Time Immunomodulation

IMMUNOSTIMULATION IN
PREVENTION

- **CITOMIX** (granuli): 5 pellets twice a day
- **GUNA-INTERFERON-GAMMA** (gocce): 20 drops twice a day
- **GUNA-INTERLEUKIN 2** (gocce) : 20 drops twice a day
- **GUNA-VIRUS**: 5 pellets twice a day
- **GUNA-FLU**: 1 dose a week

IMMUNOSTIMULATION
AND
CO-PREVENTION

- **GUNA-FLU**: 1 dose a week

Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures

Kenrie P Y Hui, Man-Chun Cheung, Ranawaka A P M Perera, Ka-Chun Ng, Christine H T Bui, John C W Ho, Mandy M T Ng, Denise I T Kuok, Kendrick C Shih, Sai-Wah Tsao, Leo L M Poon, Malik Peiris, John M Nicholls, Michael C W Chan

www.thelancet.com/respiratory **Published online May 7, 2020** **https://doi.org/10.1016/S2213-2600(20)30193-4**

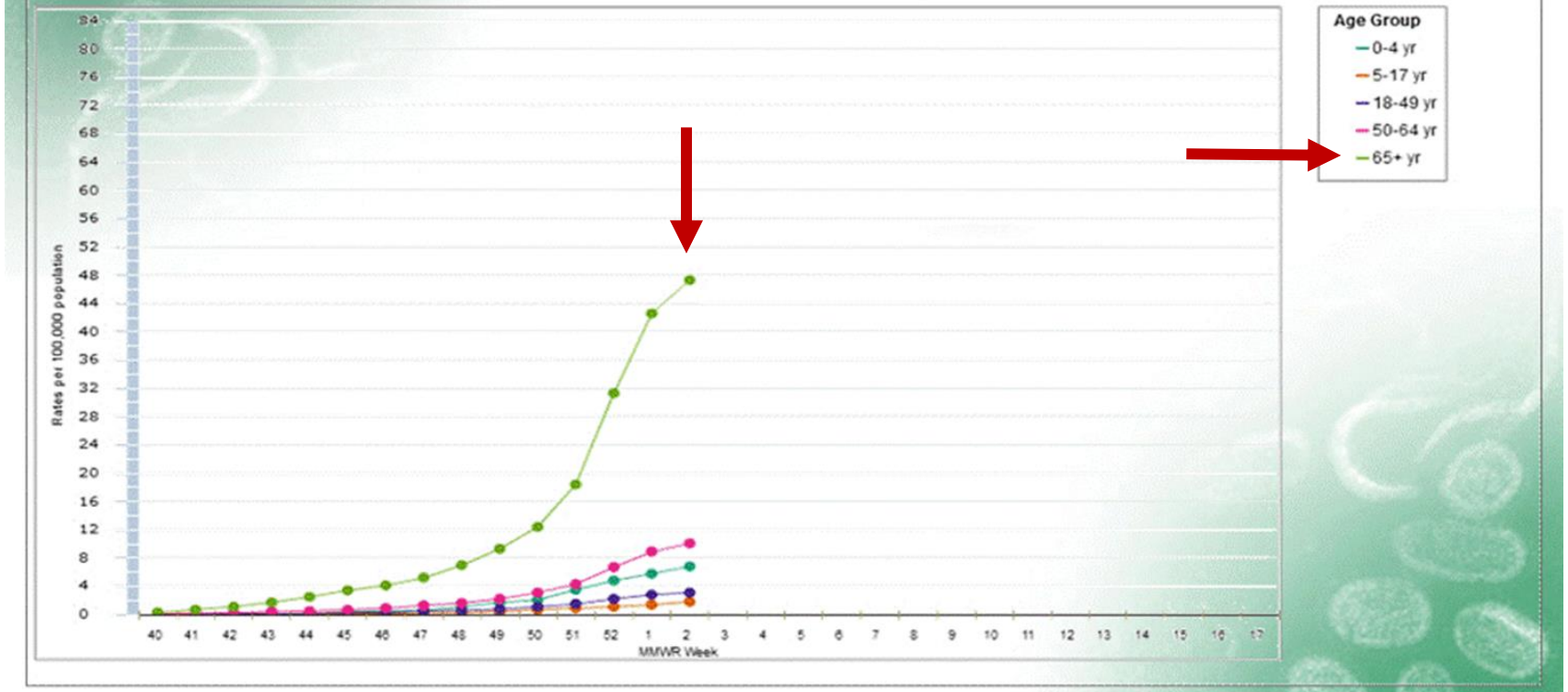
We showed that ACE2 mRNA expression was significantly upregulated in alveolar epithelial cells after influenza A virus infection, with H5N1 having a more pronounced effect than H1N1pdm in vitro. If replicated in a larger sample, this upregulation could suggest that recent exposure to influenza virus might worsen the outcome of COVID-19 through upregulation of the ACE2 receptor in human respiratory epithelium. By contrast, ACE2 expression might also offer protective effects during acute lung injury as shown for SARS.[25] Therefore, the role of ACE2 expression during influenza infection should be defined, and its implications on susceptibility to and severity of SARS-CoV-2 infection should be investigated.

FLUVIEW

A Weekly Influenza Surveillance Report Prepared by the Influenza Division

Laboratory-Confirmed Influenza Hospitalizations

Preliminary cumulative rates as of Jan 14, 2017



Problem #1

HIGHEST antigenic variability of the Orthomyxoviruses

Facing the antigenic DRIFT problem

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… but also… Problem #2

HIGHEST variability of Influenza and Parainfluenza viruses

The Journal of Infectious Diseases

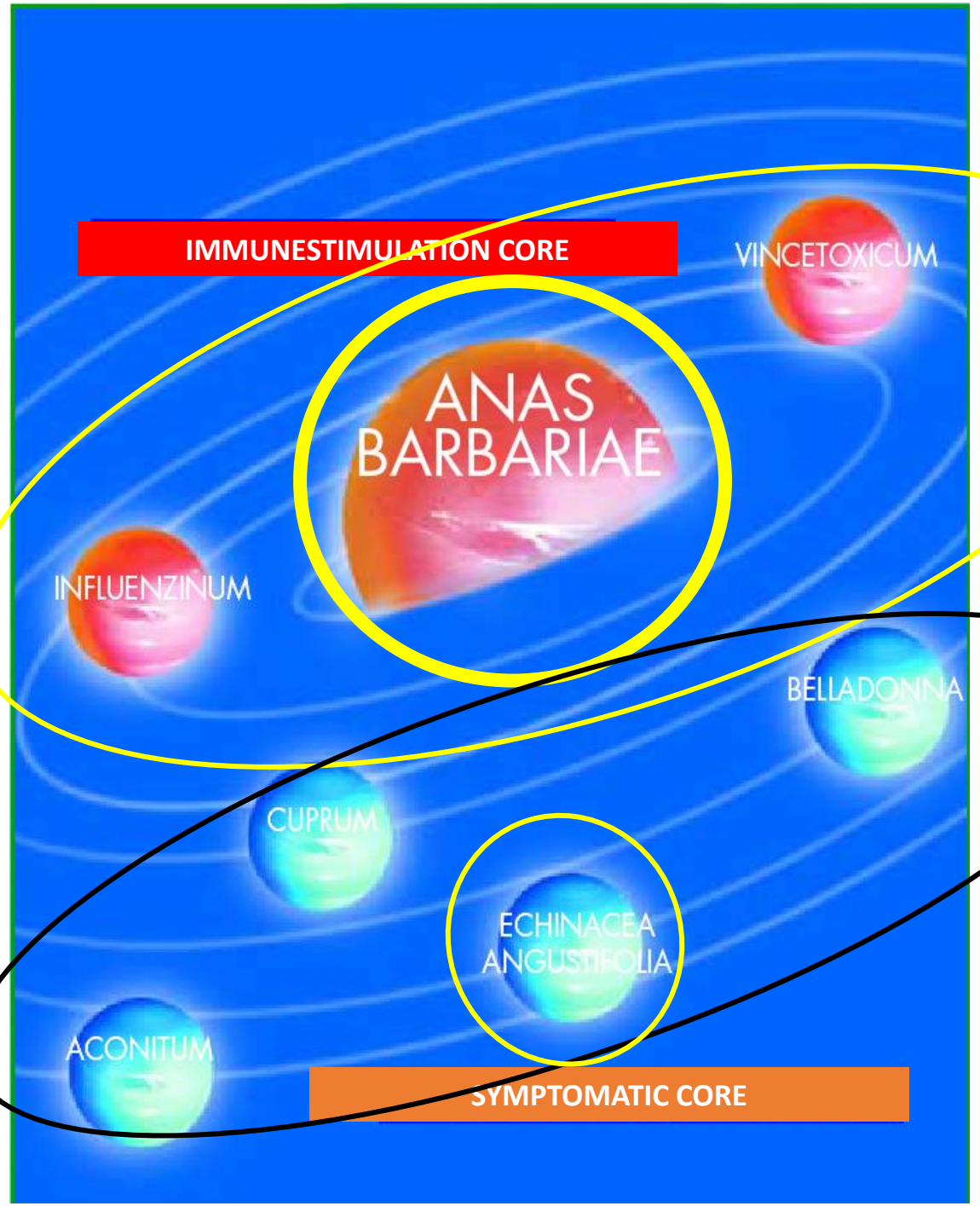
MAJOR ARTICLE

Influenza-like Illness Incidence Is Not Reduced by Influenza Vaccination in a Cohort of Older Adults, Despite Effectively Reducing Laboratory-Confirmed Influenza Virus Infections

Josine van Beek,[1] Reinier H. Veenhoven,[2,a] Jacob P. Bruin,[3] Renée A. J. van Boxtel,[1] Marit M. A. de Lange,[1] Adam Meijer,[1] Elisabeth A. M. Sanders,[1,4] Nynke Y. Rots,[1] and Willem Luytjes[1]

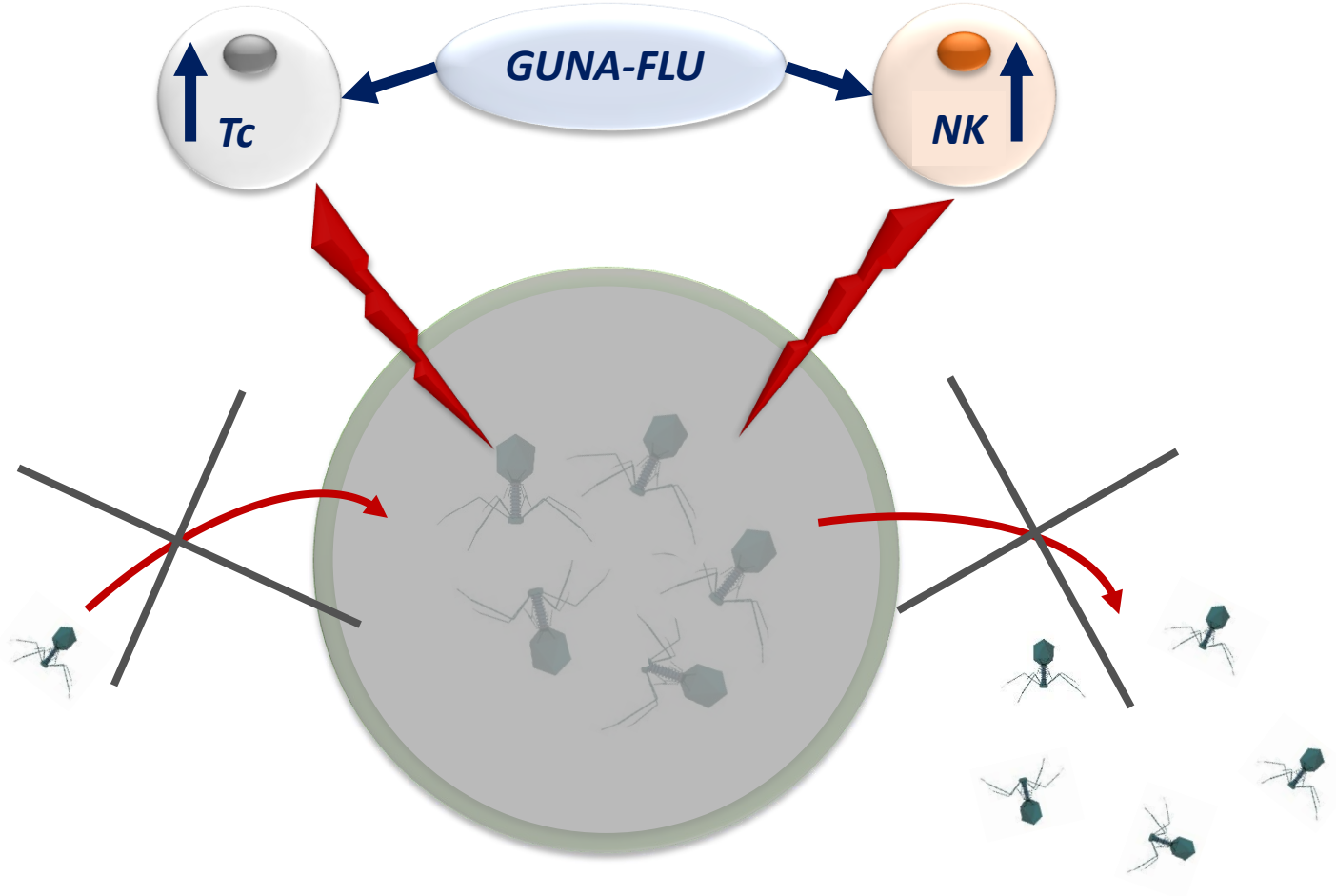
[1]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven; [2]Spaarne Gasthuis Academy, Hoofddorp; [3]Regional Laboratory for Public Health Kennemerland, Haarlem; and [4]Department of Pedriatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital/University Medical Center, Utrecht, The Netherlands

GUNA-FLU®

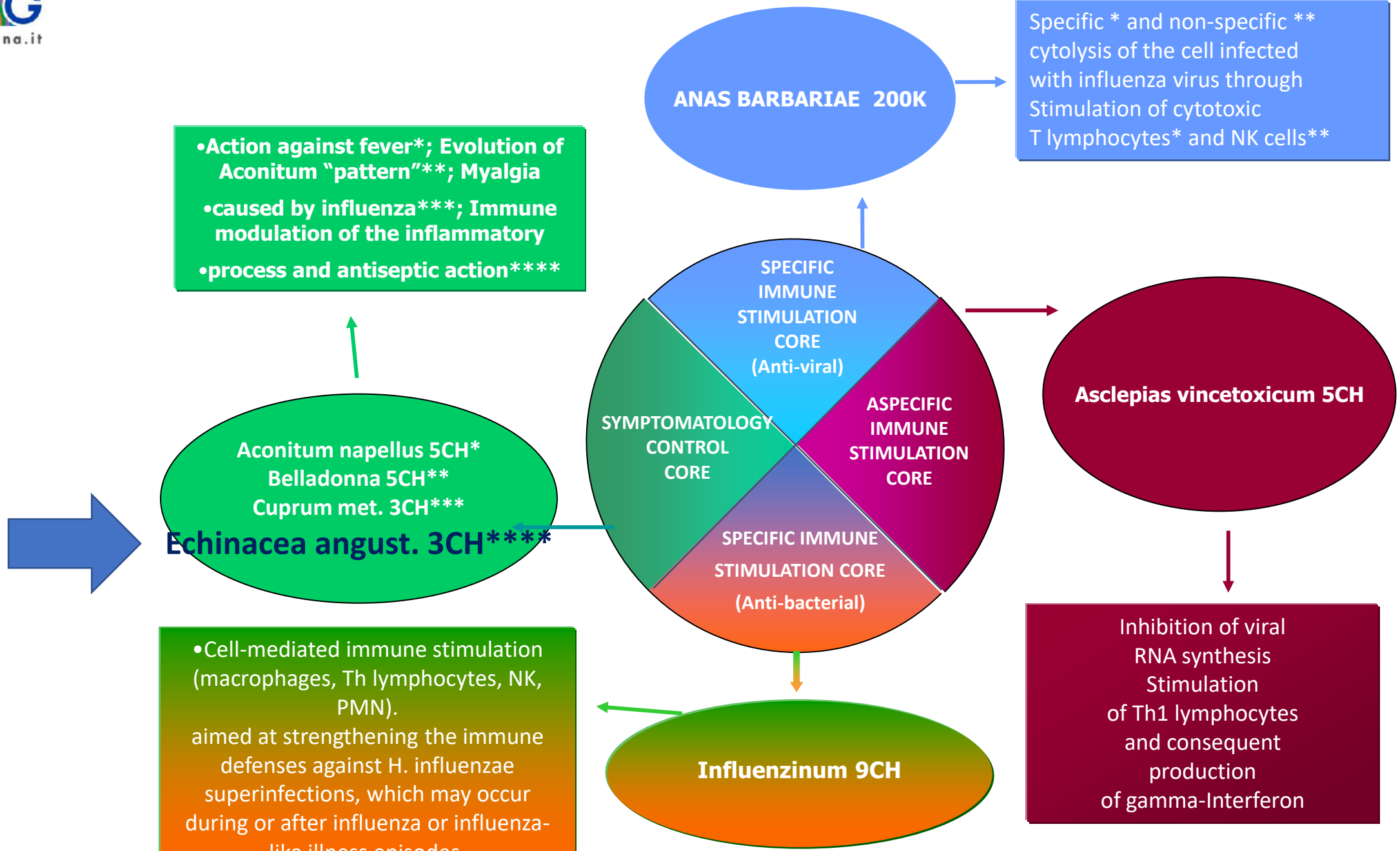


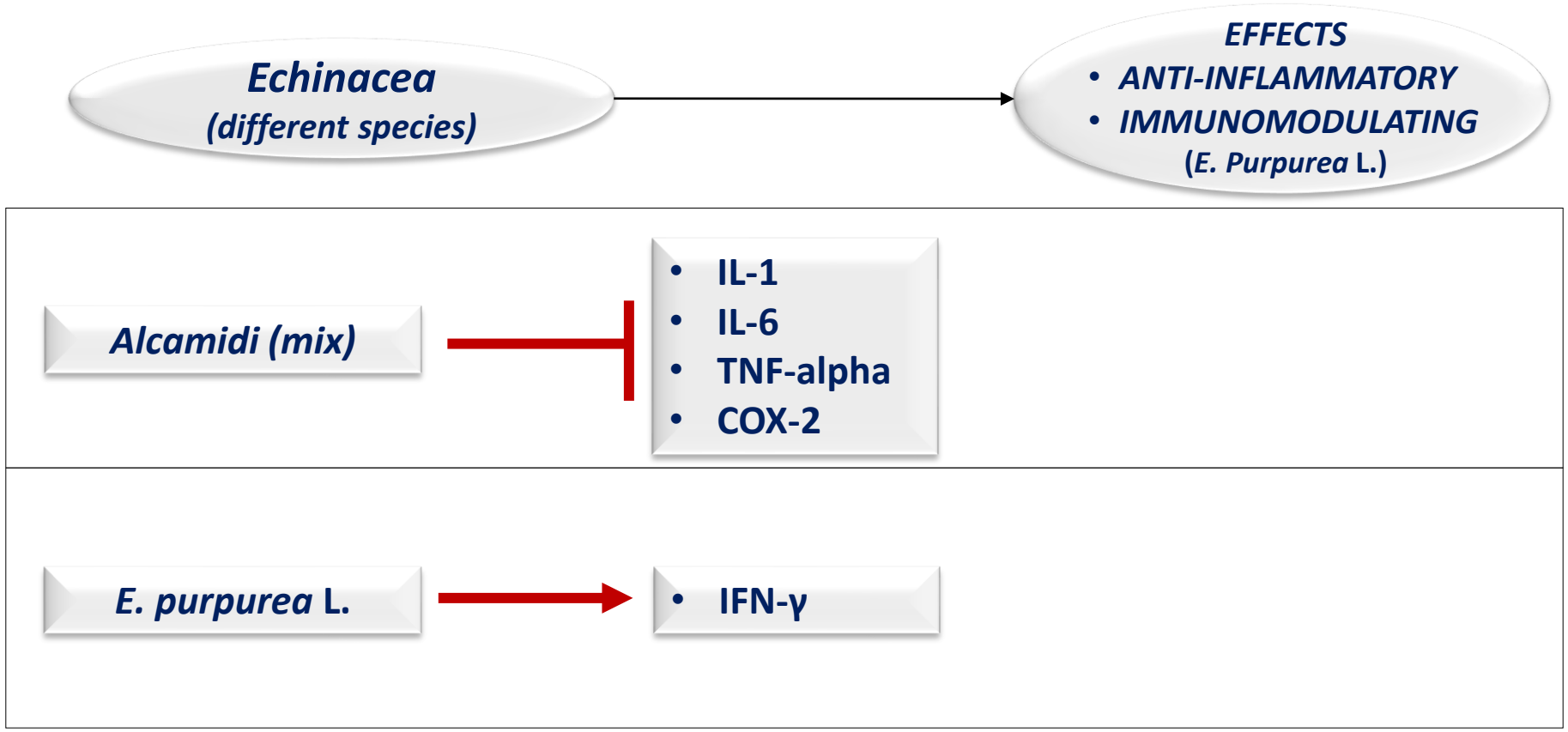
Prevention and treatment of flu and cold syndromes

MECHANISM OF ACTION OF GUNA-FLU



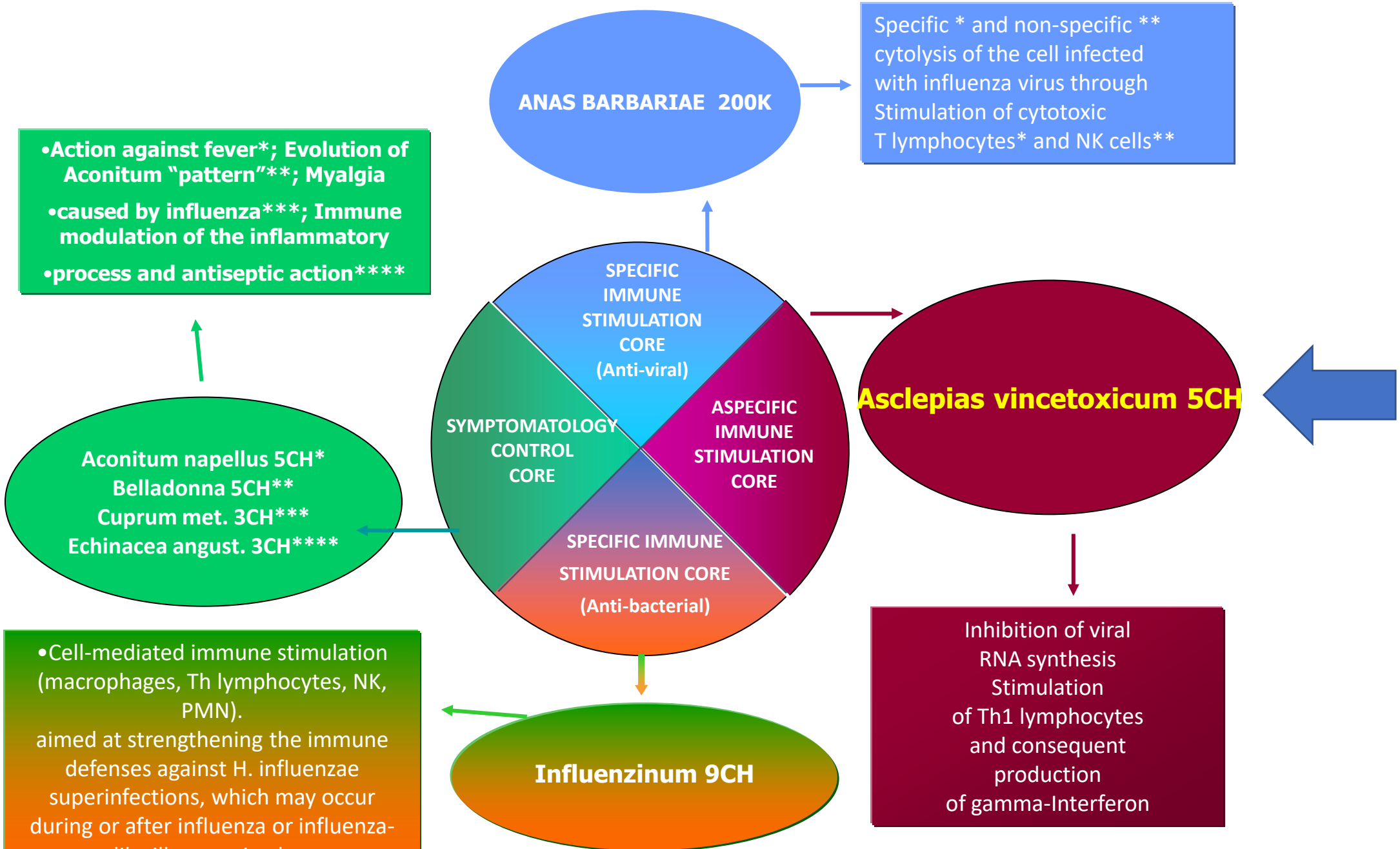
GUNA-FLU

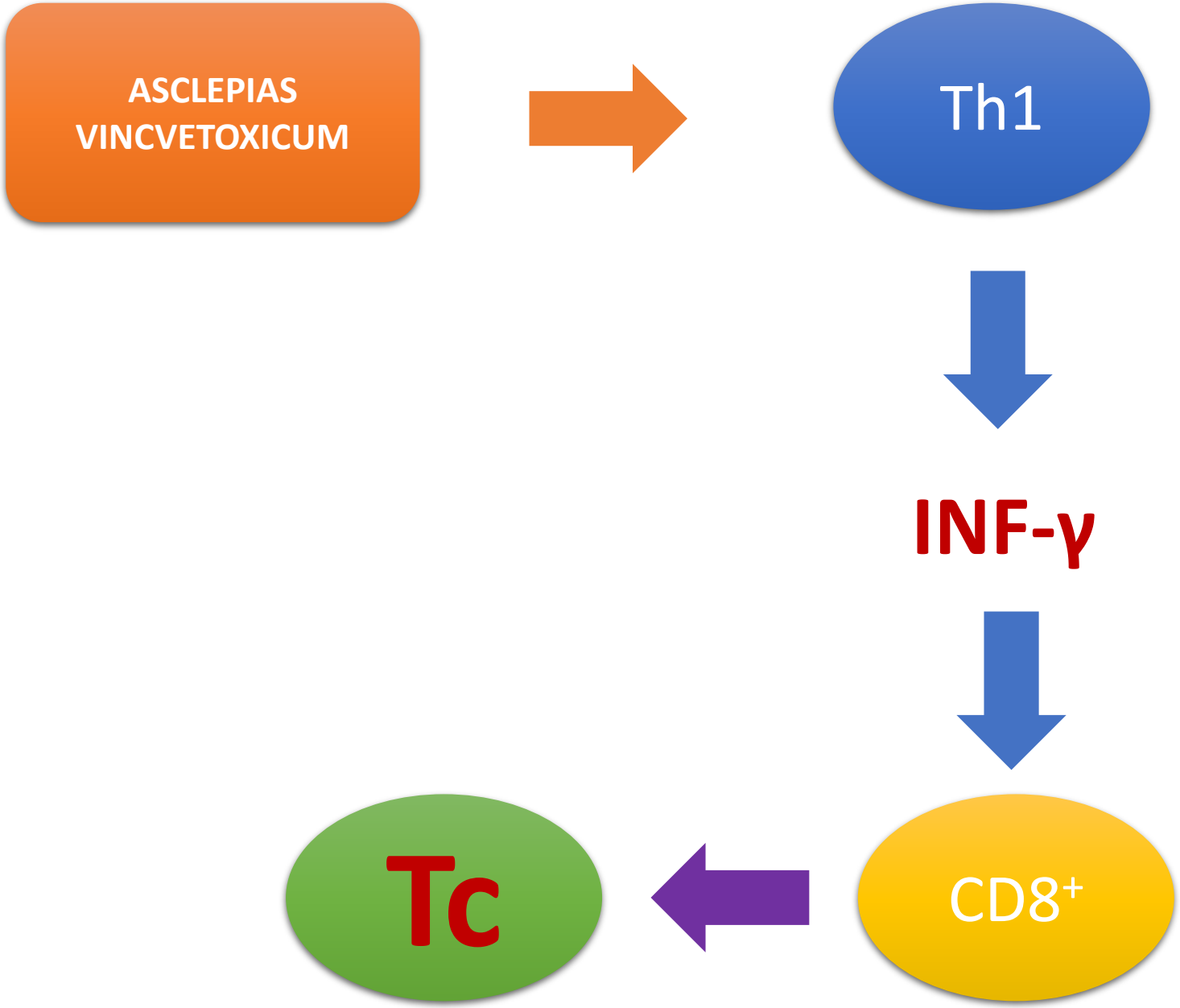




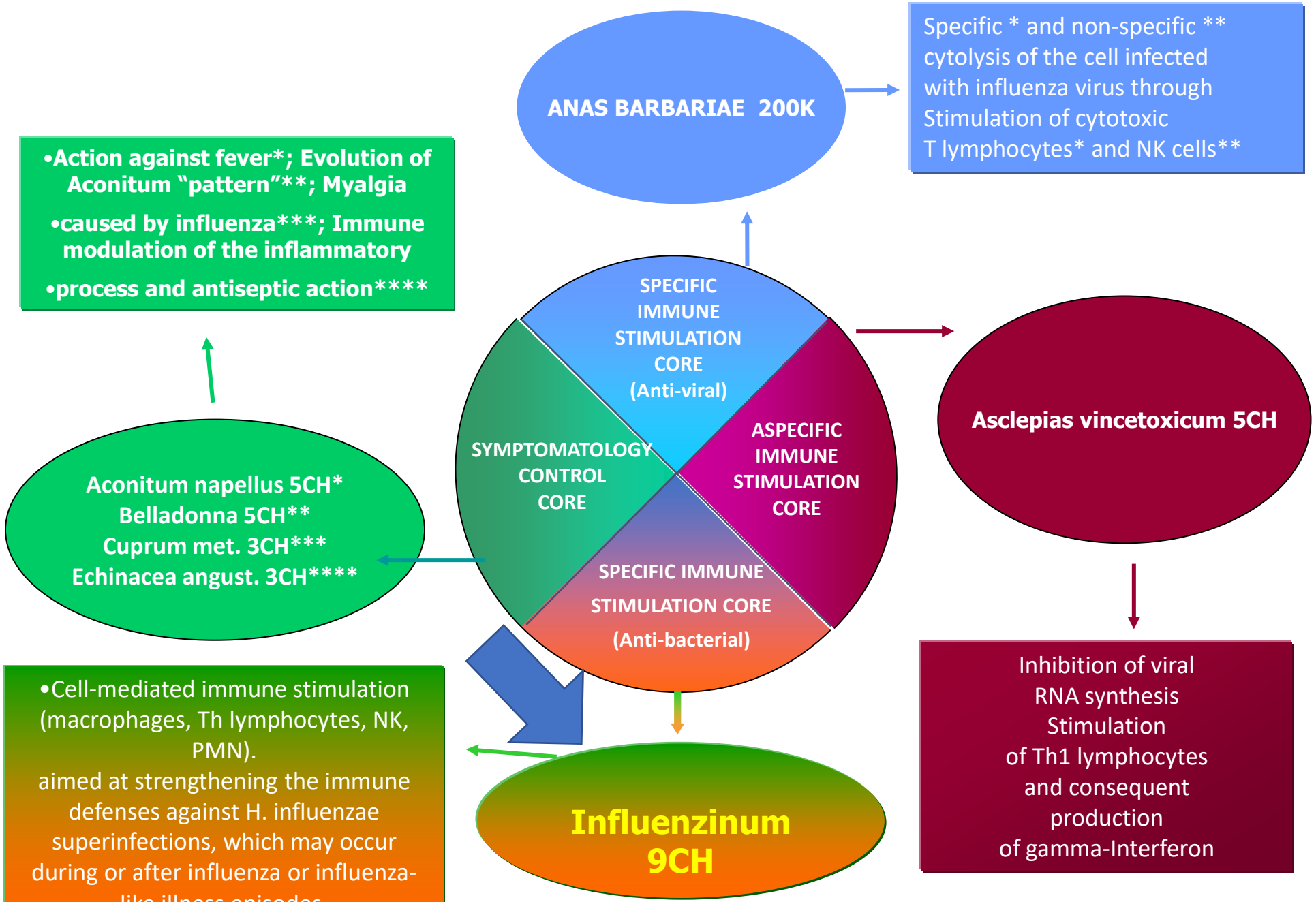
- *Hou CC, et al. Comparative metabolomics approach coupled with cell- and gene-based assays for species classification and anti-inflammatory bioactivity validation of Echinacea plants. J Nutr Biochem. 2010;21(11):1045-59.*
- *Bałan BJ, et al. The modulatory influence of some Echinacea-based remedies on antibody production and cellular immunity in mice. Cent Eur J Immunol. 2016;41(1):12-8.*
- ***Hayashi** I, et al. Effects of oral administration of **Echinacea** purpurea (American herb) on incidence of spontaneous leukemia caused by recombinant leukemia viruses in AKR/J mice. Nihon Rinsho Meneki Gakkai Kaishi. **2001;**24(1):10-20.*

GUNA-FLU




ASCLEPIAS
VINCVETOXICUM
Th1
INF-γ
CD8+
Tc

GUNA-FLU



Influencinum of GUNA-FLU is obtained from the expectorate of patients infected with influenza *and it is particularly rich of Haemophilus influenzae*

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Guna Scientific Department

GUNA®-FLU

Clinical Studies

1) COMPARATIVE EVALUATION OF GUNA®-FLU vs VACCINE FOR THE INFLUENZA SYNDROME PREVENTION IN PEDIATRICS
A prospective, multicentre, randomized, controlled study
M. Colombo, G. Rigamonti, M.L. Danza, A. Bruno
LA MEDICINA BIOLOGICA 2007/3; 3-10

2) EFFICACY OF A COMPLEX HOMOEOPATHIC MEDICINE IN THE RECURRENT RESPIRATORY INFECTIONS PREVENTION IN HIGHLY-SUSCEPTIBLE CHILDREN
A controlled, randomized study
G. Rocca, M. Colombo
ECAM (EVIDENCE BASED COMPLEMENTARY AND ALTERNATIVE MEDICINE)
- in press -

3) UPPER RESPIRATORY INFECTIONS PREVENTION IN CHILDREN WITH GUNA®-FLU
A multicentre, controlled, randomized study
C. Supino
LA MEDICINA BIOLOGICA 2002/3; 19-23

INTERNATIONAL LITERATURE REVIEW

GUNA
Omeopatia d'avanguardia

PREVENTION OVERLAP

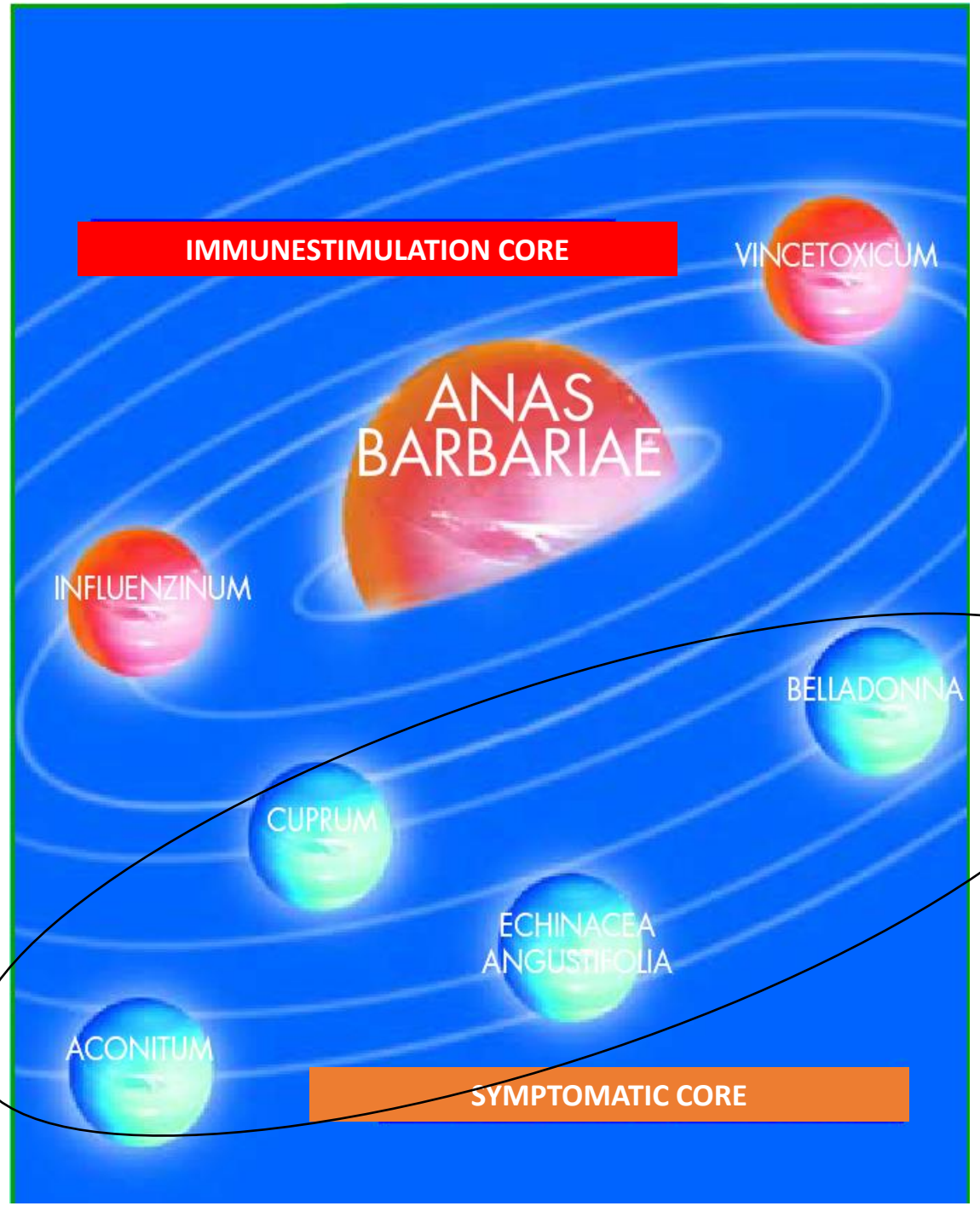
+

ANTI-FLU VACCINE

=

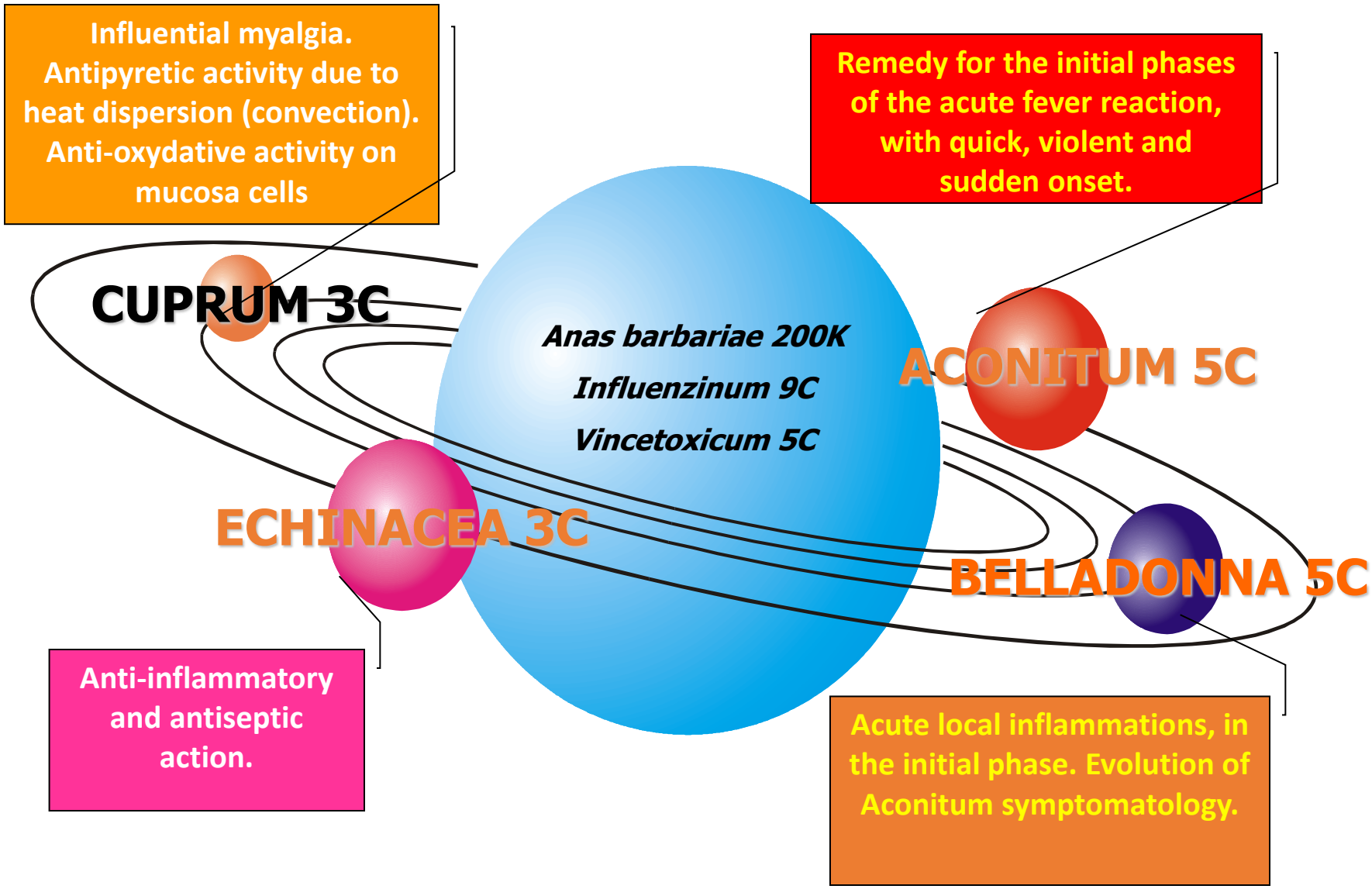
BY-PASSING THE ANTIGENIC DRIFT OF THE INFLUENZA VIRUS

GUNA-FLU®



Prevention and treatment of flu and cold syndromes

SYMPTOMATIC REMEDIES CORE



GUNA-FLU

Directions

- **SEASONAL PREVENTION: one dose once a week for 6-8 weeks to be repeated after 2 weeks**

- **TREATMENT OF THE ACUTE SYMPTOMATOLOGY (in the first 36 hours from the onset): one dose every 6 hours until acute symptoms disappear**

guna.it

SUDDIVISIONE DEI PAZIENTI PER TIPO DI TERAPIA	
Terapia	**N° pazienti**
Gruppo A – OMEOGRIPHI®	78 (43 F, 35 M)
Gruppo B – Paracetamolo	81 (45 F, 36 M)

Riferimento bibliografico

ARRIGHI A. – Omeogriphi® *vs* paracetamolo nel trattamento della Sindrome influenzale.
– Studio clinico prospettico controllato
La Med. Biol., **2013**/4; 3-12.

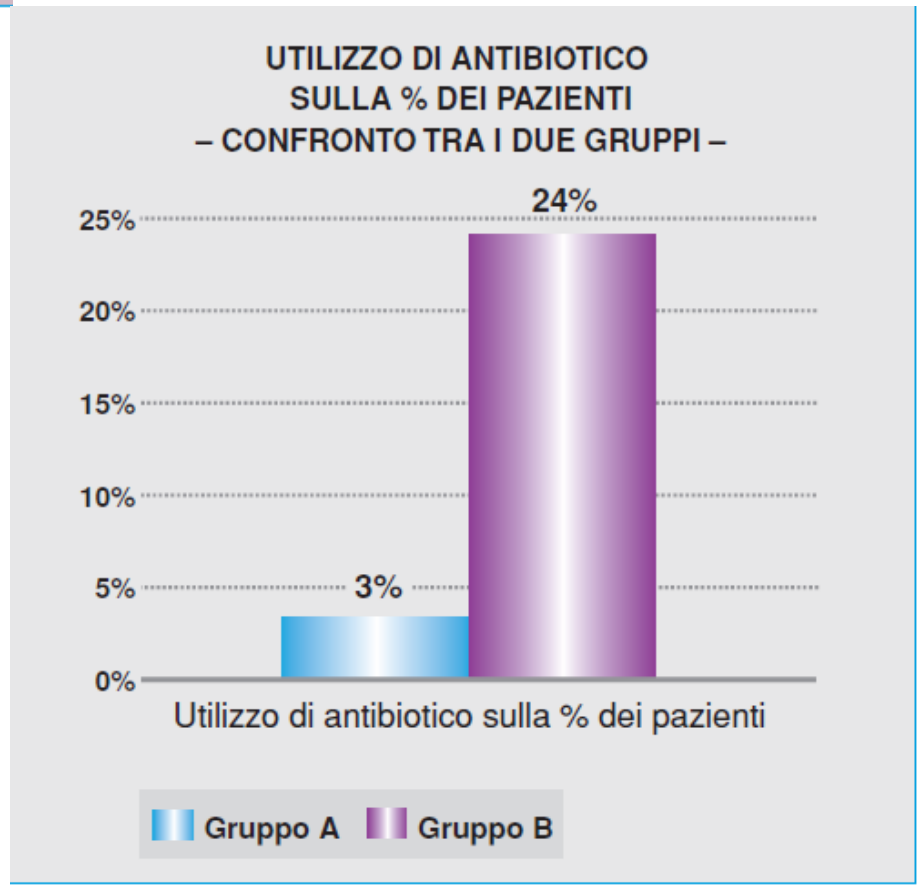
SINTOMI GENERALI	PUNTEGGIO MEDIO	SINTOMI RESPIRATORI	PUNTEGGIO MEDIO	SINTOMI GASTRO-INTESTINALI	PUNTEGGIO MEDIO
• CEFALEA • MALESSERE GENERALE • SENSAZIONE DI FEBBRE (brividi, sudorazione) • DOLORI MUSCOLARI		• TOSSE • FARINGODINIA • CONGESTIONE NASALE • RAUCEDINE • AFONIA • TORACODINIA		• NAUSEA • VOMITO • DIARREA • DOLORI ADDOMINALI	

PUNTEGGIO	SIGNIFICATO CLINICO
0	Assenza di sintomi
1	Sintomi di gravità lieve
2	Sintomi di gravità moderata
3	Sintomi di gravità alta

FIG. 2

RISOLUZIONE DEL SINTOMO FEBBRE
dopo 24-48-72 ore

	Dopo 24h (percentuale pazienti)	Dopo 48h (percentuale pazienti)	Dopo 72h (percentuale pazienti)
Gruppo A – OMEOGRIPHI®	25%	56%	89%
Gruppo B – Paracetamolo	23%	49%	76%



Space-Time Immunomodulation

IMMUNOSTIMULATION IN **PREVENTION**

- CITOMIX (granuli): 5 pellets twice a day
- GUNA-INTERFERON-GAMMA (gocce): 20 drops twice a day
- GUNA-INTERLEUKIN 2 (gocce) : 20 drops twice a day
- GUNA-VIRUS: 5 pellets twice a day

- GUNA-FLU: 1 dose a week

IMMUNOSTIMULATION IN THE **EARLY STAGE OF THE INFECTION**
AND
EARLY STAGE ANTINFLAMMATORY THERAPY

- **CITOMIX** (granuli): 10 pellets 2-3 days a day
- **GUNA-INTERLEUKIN 2** (gocce): 20 drops 4-6 times a day
- **GUNA-VIRUS**: 5 pellets 4-6 times a day

- **GUNA-ANTIL IL 1**: **20 drops 6-8 times a day for 2 to 6 days**

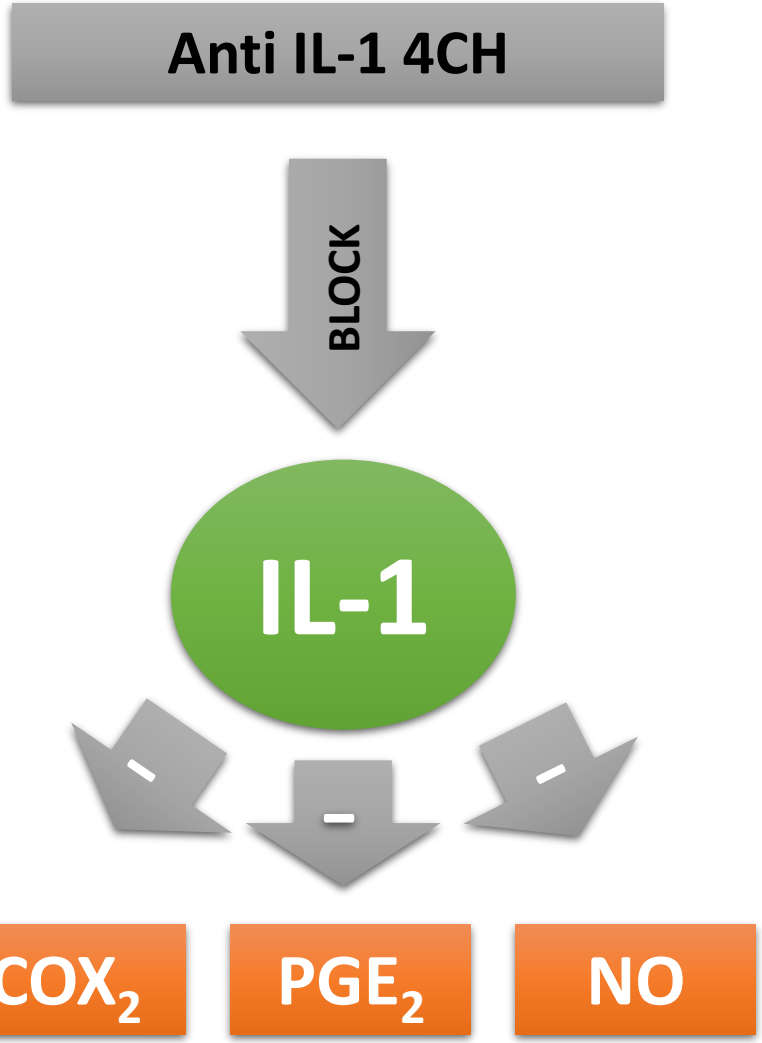
A novel, systemic, approach to
The first stage of the inflammatory phase of an infection

DIRECTIONS AND WAY OF ADMINISTRATION

Guna-Anti IL 1: 20 drops 10-12 times a day for a short-medium time (half dosage for children below 6 years)

Sublingual administration directly under the tongue or in a little water, preferibly far from meals.

Anti Interleukins-1 (α; ß) **act** as **NSAIDs, cortisone and, in part, as salicylates** , without the negative side effects caused by these allopathic medicines.



Space-Time Immunomodulation

IMMUNOSTIMULATION	• CITOMIX (granuli) • GUNA-INTERFERON-GAMMA (gocce) • GUNA-INTERLEUKIN 2 (gocce) • GUNA-VIRUS • GUNA-FLU
IMMUNOSTIMULATION AND ANTINFLAMMATORY THER.	• GUNA-INTERELUKIN 2 (gocce) • CITOMIX (granuli) • GUNA-ANTI IL 1 (gocce) • GUNA-FLAM (fiale e compresse)
ANTINFLAMMATORY THER.	• **GUNA-FLAM**: 20 drops 4-6 times a day
CONVALESCENCE AND PREVENTION OF RELAPSES	• GUNA-INTERLEUKIN 7 (gocce) • CITOMIX (Granuli)

A novel, systemic, approach to
The second stage of the inflammatory phase of an infection

DIRECTIONS AND WAY OF ADMINISTRATION

Guna-Flam: 20 drops twice to four times a day a day (half dosage for children below 6 years)

Sublingual administration directly under the tongue or in a little water, preferibly far from meals.

Ingredients

Aconitum napellus 6X/12X/30X/200X
Anti interleukin 1 alpha 4C
Apis mellifica 6X/12X/30X/200X
Belladonna 6X/12X/30X/200X
Beta-Endorphin 6X
Bryonia alba 6X/12X/30X/200X
Citricum acidum 3X
Conjunctiva tissue, Porcine 12X/30X/200X
Copper gluconate 4X
Ferrum phosphoricum 6X/12X/30X/200X
Hepar sulphuris calcareum 6X/12X/30X/200X
Hypophysis, Porcine 200X
Interleukin 10 4C
Melatonin 4C
Natrum pyruvicum 3X
Phytolacca decandra 6X/12X/30X/200X
Pineal gland, Porcine 6X
Pyrogenium 30X/200X
Transforming Growth Factor 1 beta 4C

Space-Time Immunomodulation

IMMUNOSTIMULATION	• CITOMIX (granuli) • GUNA-INTERFERON-GAMMA (gocce) • GUNA-INTERLEUKIN 2 (gocce) • GUNA-VIRUS • GUNA-FLU
IMMUNOSTIMULATION AND ANTINFLAMMATORY THER.	• GUNA-INTERELUKIN 2 (gocce) • CITOMIX (granuli) • GUNA-ANTI IL 1 (gocce) • GUNA-FLAM (fiale e compresse)
ANTINFLAMMATORY THER.	• GUNA-FLAM: 20 drops 4-6 times a day
CONVALESCENCE AND PREVENTION OF RELAPSES	• **GUNA-INTERLEUKIN 7** (gocce): 20 drops twice a day for 2-4 months • **CITOMIX** (Granuli) : 5 pelltes one a day for 2-4 months (preferibly in association with **Guna-Interleukin 2**)

A novel, systemic, approach to *The post-active infection phase*

DIRECTIONS AND WAY OF ADMINISTRATION

Guna Interleukin-7: 20 drops twice a day for 2 to 4 months (half dosage for children below 6 years)

Sublingual administration directly under the tongue or in a little water, preferibly far from meals.

INTERLEUKIN-7 IS STRONGLY ACTIVE ON THE IMMUNOLOGICAL MEMORY

- **Stimulates the proliferation of all cells of the lymphoid line (B cells, T cells, NK)**
- It has **strong anti-apoptotic activities** and therefore prolongs the life of these cell populations
- It induces differentiation in the Th1 direction, by up-regulation of IL-2 and IFN-γ
- In synergy with IL-12 it favors the differentiation of T-naive into Th1, their proliferation and stabilization
- **It stimulates cytolytic activity through the induction of the IFN-γ synthesis**
- **It stimulates immunological memory ***

- ***** Memory T lymphocytes are responsible for the increased speed and effectiveness with which immune responses occur when an organism comes into contact with the same antigen again.**

Space-Time Immunomodulation

IMMUNOSTIMULATION IN PREVENTION

- CITOMIX (granuli): 5 pellets twice a day
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IMMUNOSTIMULATION IN THE EARLY STAGE OF THE INFECTION AND ANTINFLAMMATORY THERAPY

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ANTINFLAMMATORY THERAPY

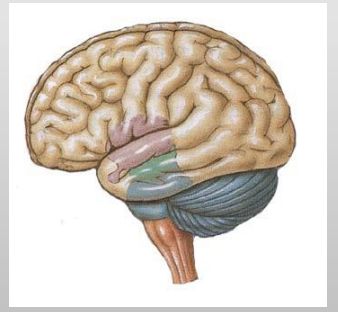
- GUNA-FLAM: 20 drops 4-6 times a day

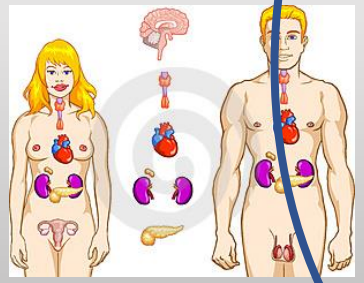
CONVALESCENCE AND PREVENTION OF RELAPSES

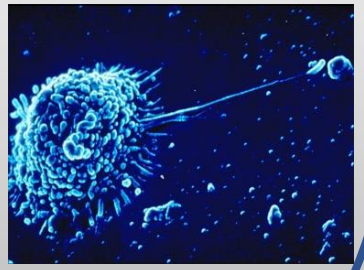
- GUNA-INTERLEUKIN 7 (gocce): 20 drops twice a day for 2-4 months
- CITOMIX (Granuli): 5 pelltes one a day for 2-4 months (preferibly in association with Guna-Interleukin 2)

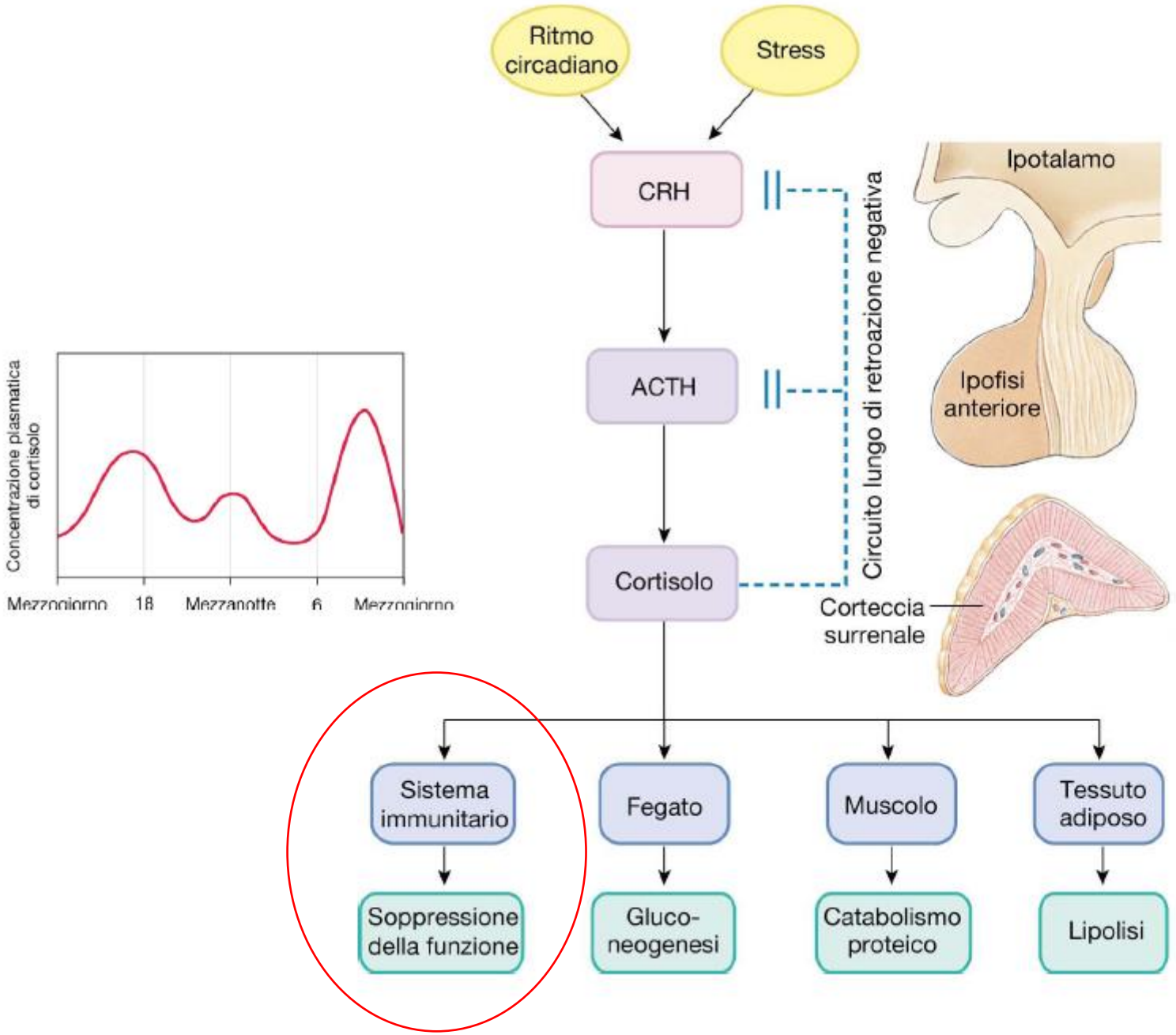
STRESS

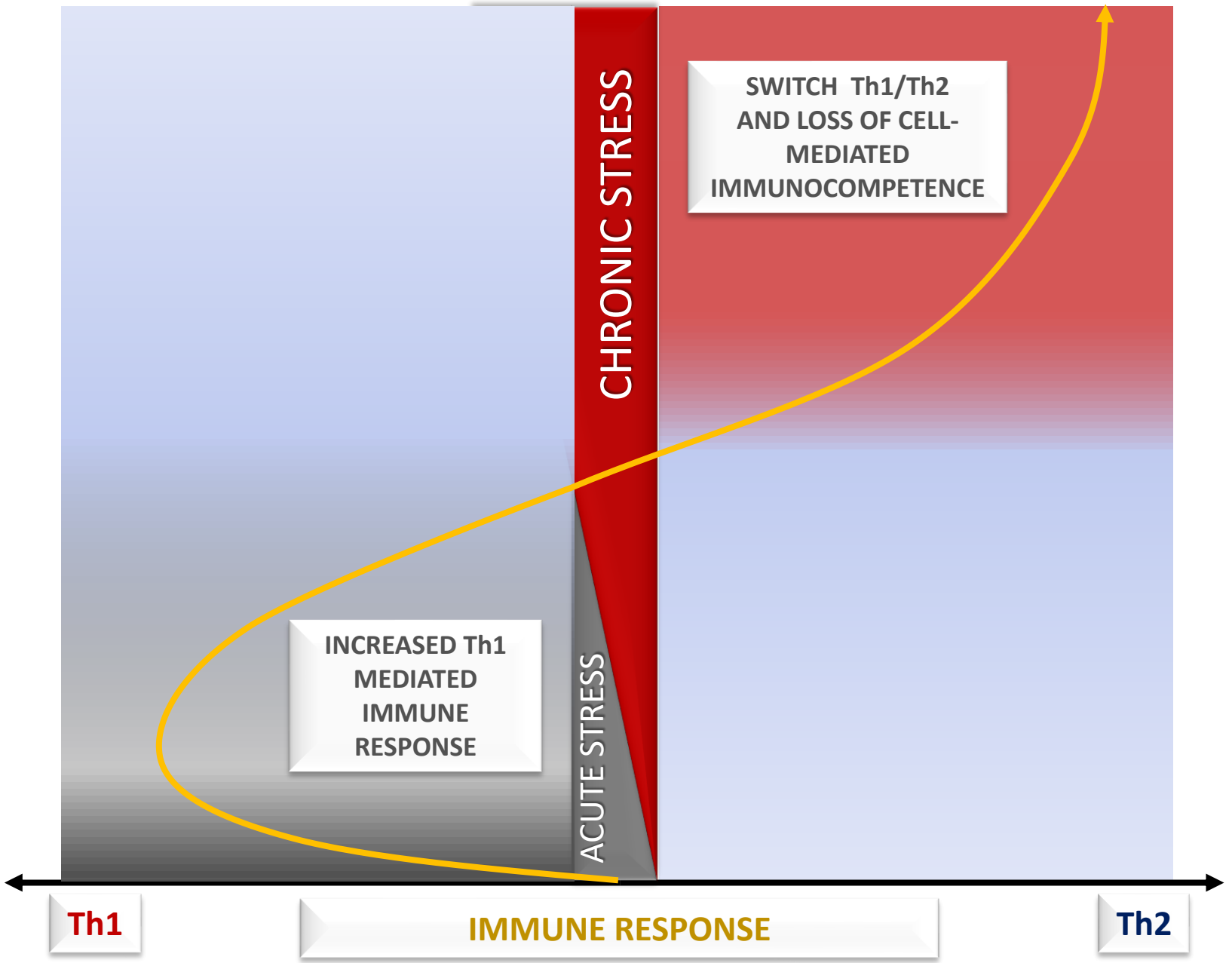
PN E I



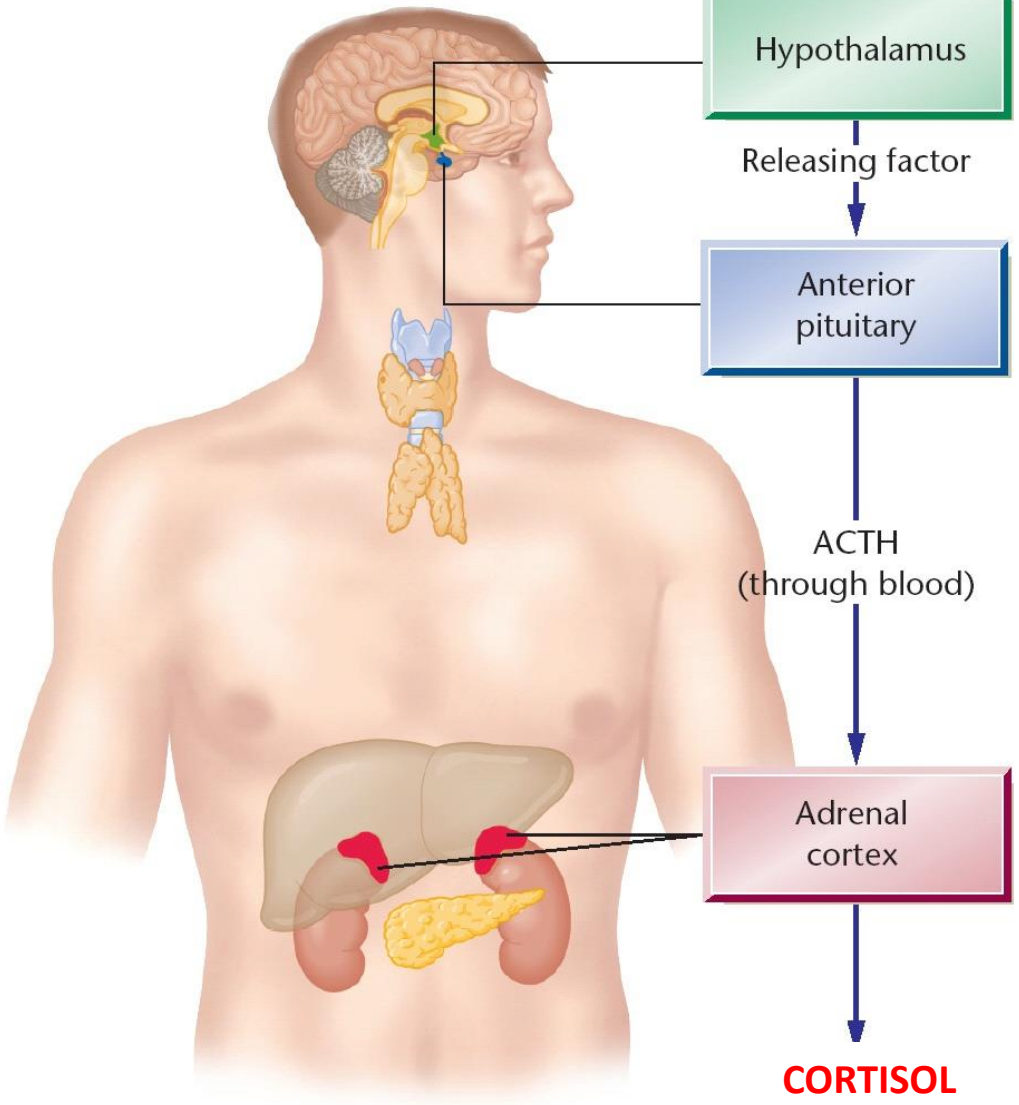





Ritmo circadiano
Stress
CRH
ACTH
Cortisolo
Circuito lungo di retroazione negativa
Ipotalamo
Ipofisi anteriore
Corteccia surrenale
Concentrazione plasmatica di cortisolo
Mezzogiorno
18
Mezzanotte
6
Mezzogiorno
Sistema immunitario
Fegato
Muscolo
Tessuto adiposo
Soppressione della funzione
Gluco-neogenesi
Catabolismo proteico
Lipolisi



STRESS AXIS



Hypothalamus

Releasing factor

Anterior pituitary

ACTH (through blood)

Adrenal cortex

CORTISOL

Tc

CD8+

Not correct stimulation of

Reduction in IFN-γ

Th2

Th1

DOWN-REGULATES Th1

LETTER

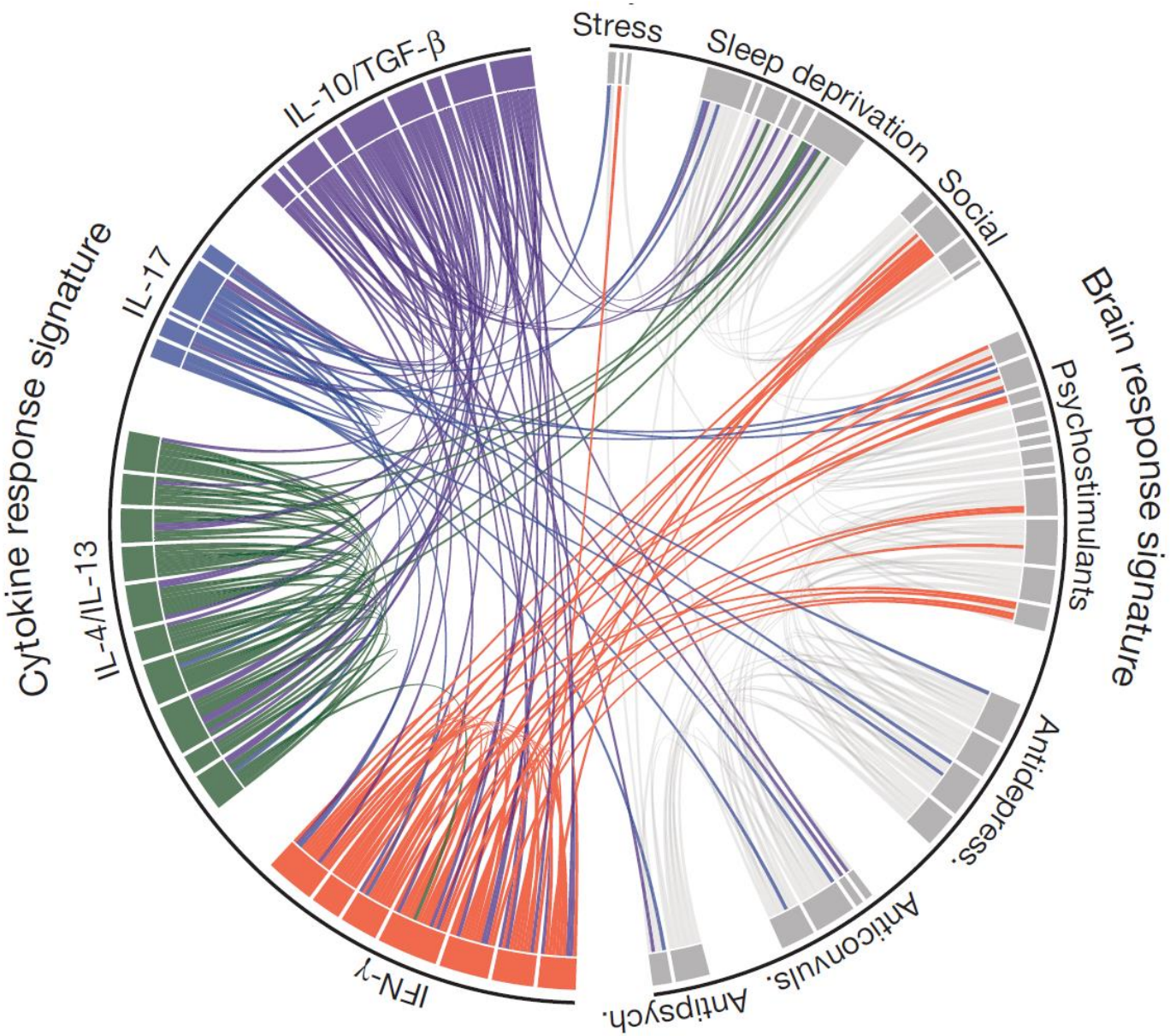
doi:10.1038/nature18626

Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour

Anthony J. Filiano[1,2], Yang Xu[3], Nicholas J. Tustison[4], Rachel L. Marsh[1,2], Wendy Baker[1,2], Igor Smirnov[1,2], Christopher C. Overall[1,2], Sachin P. Gadani[1,2,5,6], Stephen D. Turner[7], Zhiping Weng[8], Sayeda Najamussahar Peerzade[3], Hao Chen[8], Kevin S. Lee[1,2,5,9], Michael M. Scott[5,10], Mark P. Beenhakker[5,10], Vladimir Litvak[3]* & Jonathan Kipnis[1,2,5,6]*

guna.it

Transcriptome analysis



A novel, systemic, approach to *viral infections protection*

DIRECTIONS AND WAY OF ADMINISTRATION

- **Guna Interferon gamma: 20 drops twice a day for 2 to 4 months (half dosage for children below 6 years)**
- **Guna Interferon alfa: 20 drops twice a day for 2 to 4 months (half dosage for children below 6 years)**

Sublingual administration directly under the tongue or in a little water, preferibly far from meals.

INTERFERONE GAMMA E ALFA SONO PARTICOLARMENTE ATTIVI SULL'INNESCO DELLA RISPOSTA CITOLITICA

- **IFN-gamma** is able to activate a cell-mediated immune response (IFN-gamma stimulates CD8 + to differentiate into cytotoxic T effector cells) ideal against viruses. The Tc, in fact, operate the non-specific cytolysis of the cell infected with the virus (the Natural Killer - NK cells - instead, operate the specific cytolysis).

- **Interferon-alpha** (in some papers alpha seems to be favored over gamma; it is interesting how Interferon-alpha prevents the virus from penetrating through the viropexy mechanism, used by many viruses, into the cells not yet infected

*[**IFN-gamma** is also used by the body for the synthesis (conversion) into IFN-alpha (it is a bit like the mechanism of reciprocity between hormone T4 and T3, where T4 is the precursor of the hormone T3, true effector of the activity thyroid]*

CITOMIX

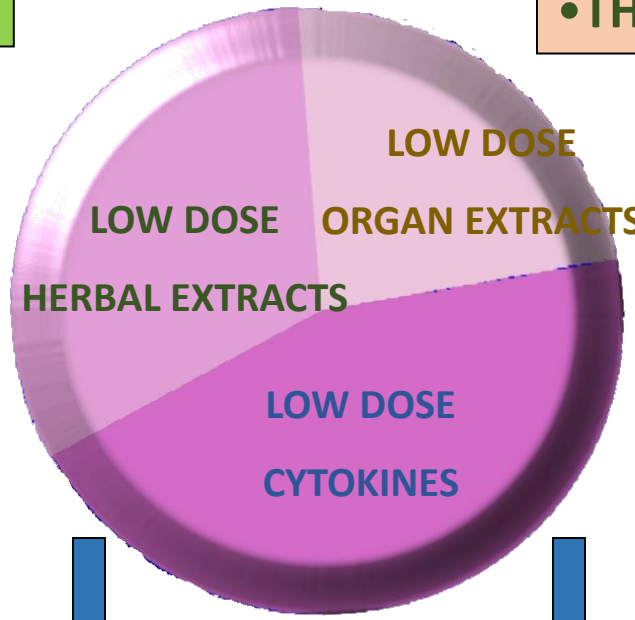
- VACCINIUM VITIS
- ANANASSA SATIVA
- HYDROCOTYLE ASIATICA (CENTELLA ASIATICA)

ANTINFLAMMATORY AND ANTIOXIDANT ACTION (*Vaccinium vitis*),

RES STIMULATION (*Ananassa sativa*),

ANTINFIAMMATORY ACTION (*Hydrocotyle asiatica*)

- VASA LYMPHATICA SUIS
- MEDULLA OSSIS SUIS
- THYMULINE



TARGETED ANTINFLAMMATORY ACTION; STIMULATION OF IMMUNOCOMPETENT TISSUES

- IL-4
- IL-2

B PROLIFERATION AND APC BOOSTING (IL-4); B, T AND NK STIMULATION (IL-2)

- GCSF
- IL1-beta
- **INF -gamma**
- IL-6

ONSET OF THE IMMUNE RESPONSE

The bag of tools

Thank you very much

nature medicine

BRIEF COMMUNICATION

https://doi.org/10.1038/s41591-020-0868-6

SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes

Waradon Sungnak[1]✉, Ni Huang[1], Christophe Bécavin[2], Marijn Berg[3,4], Rachel Queen[5], Monika Litvinukova[1,6], Carlos Talavera-López[1], Henrike Maatz[6], Daniel Reichart[7], Fotios Sampaziotis[8,9,10], Kaylee B. Worlock[11], Masahiro Yoshida[11], Josephine L. Barnes[11] and HCA Lung Biological Network*✉

We investigated SARS-CoV-2 potential tropism by survey- associated with SARS-CoV-2 pathogenesis at cellular resolution