

Female immunsystem and pregnancy

Interventional tolerance
induction by Omega-3-fatty
acids and *Vitamin-D*

Types of Transplantations

Transplantation

The grafting of tissue, usually from one individual to another.

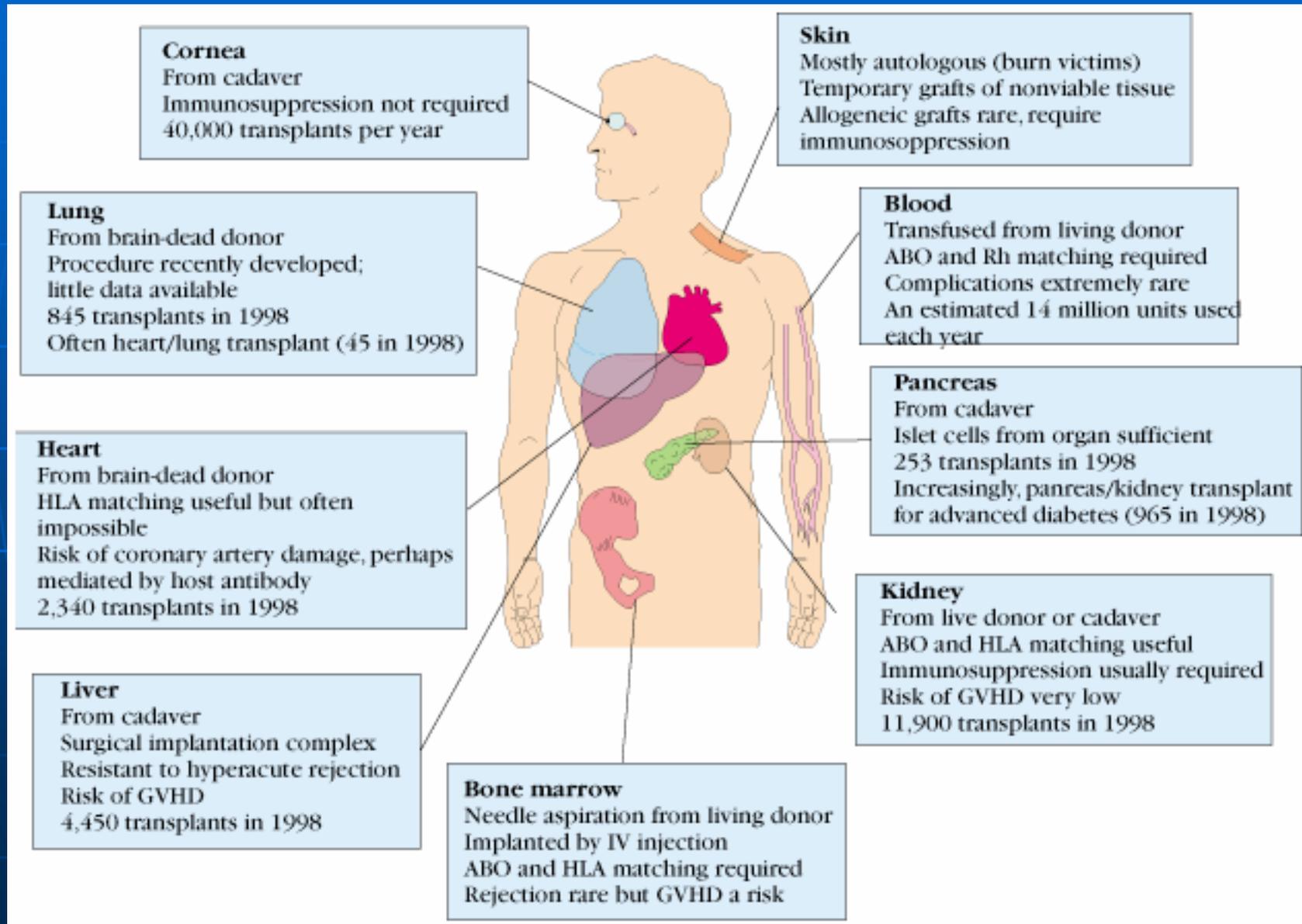
Autograft - to another site on the same individual

Isograft - to a genetically identical individual
(iso/syngeneic)

Allograft - to a genetically disparate member of the same species
(allogeneic)

Xenograft - to a different species
(xenogeneic)

Transplanted tissues



Immunogenicity of cells, tissues and organs

Organs

allogeneic
(xenogeneic) ++++

Cells

autolog
(allogeneic)
(xenogeneic) ++

Tissue

autolog
(allogeneic)
(xenogeneic) +++

Pregnancy



In solid tissue transplantation, there are three major types of graft rejection:

hyperacute rejection:

preformed antibodies
minutes - days

acute rejection:

T-cells
days - weeks, also late

chronic rejection

T-cells, antibodies
macrophages, cytokines
(weeks -) months - years

Reception of Signals

harmless

out of interest

dangerous



Tolerance

- active
- prevents overreaction
- allows co-existence
- allows pregnancy

Ignorance

- passive
- not interactive
- instable

Aggression

- active
- protective, if regulated
- self-destruction possible

Immunogenicity of cells, tissues and organs

Why, and how will allogenic
(under certain conditions
also autologous) cells,
tissues and organs be
rejected

Immunogenicity of cells, tissues and organs

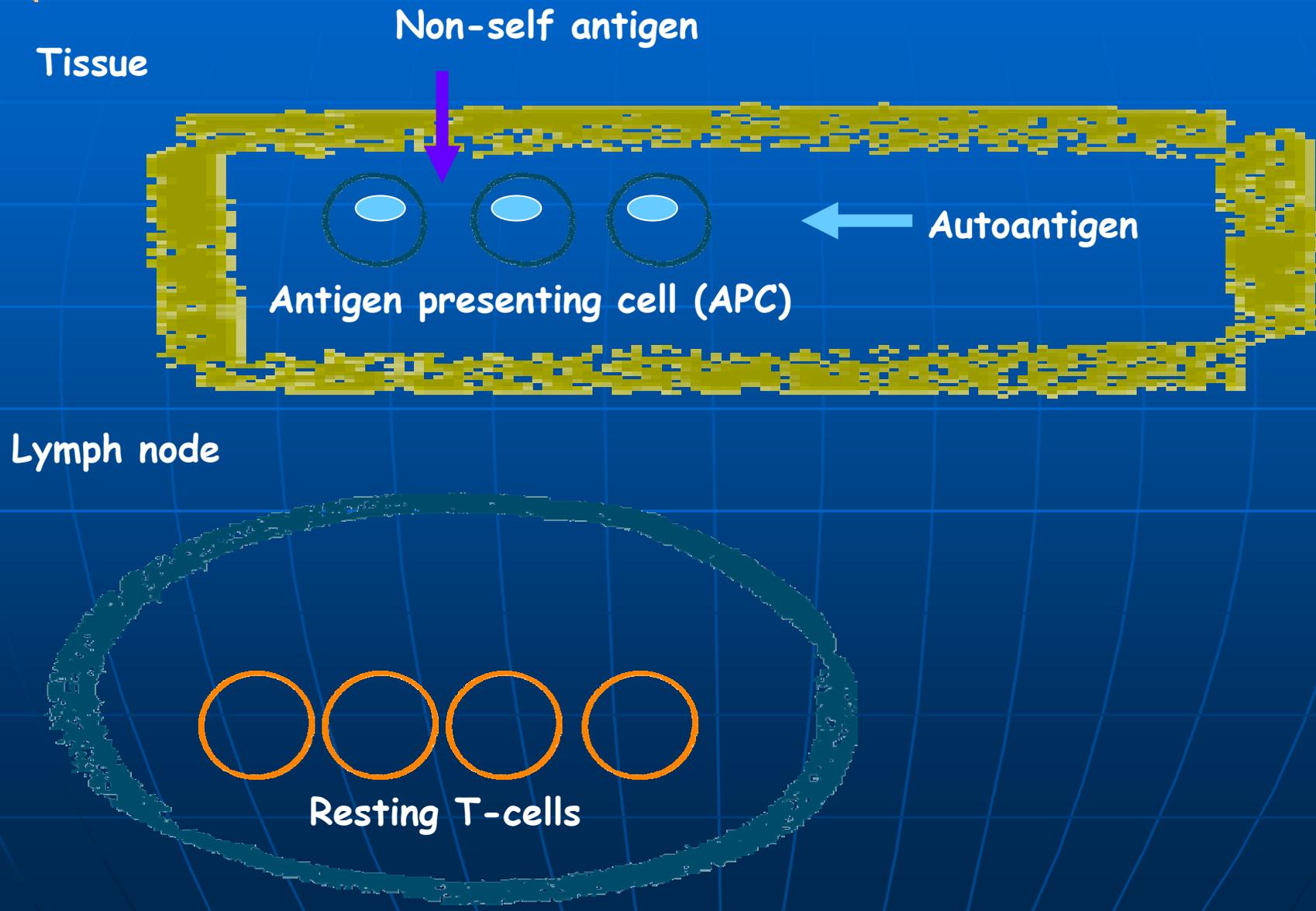
Conventional conception:

The immune system can only discriminate between „non-self“ and „self“

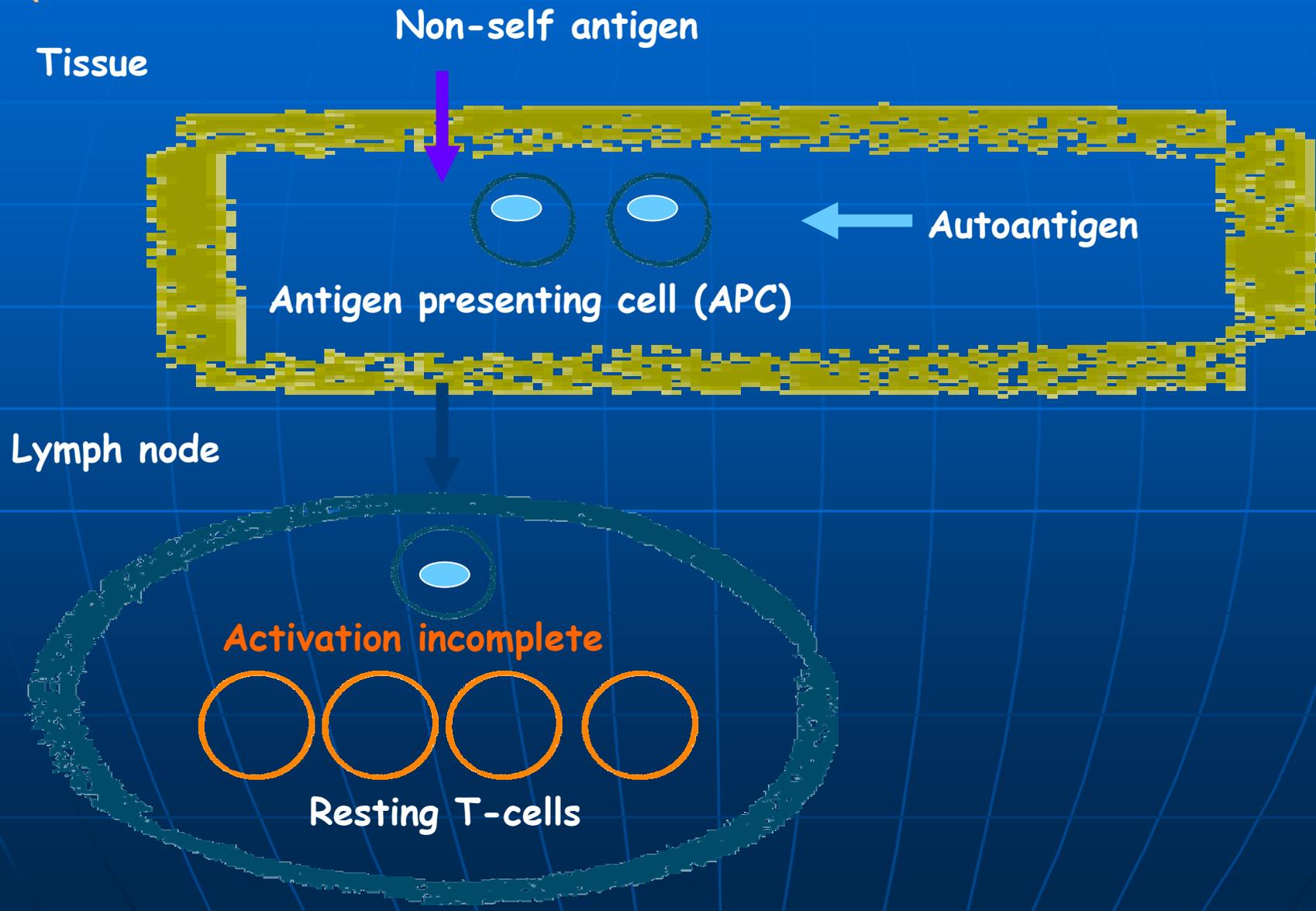
Actual conception:

The immune system can discriminate between „self“, „non-self AND dangerous“ and „non-self and NOT dangerous = tolerance“

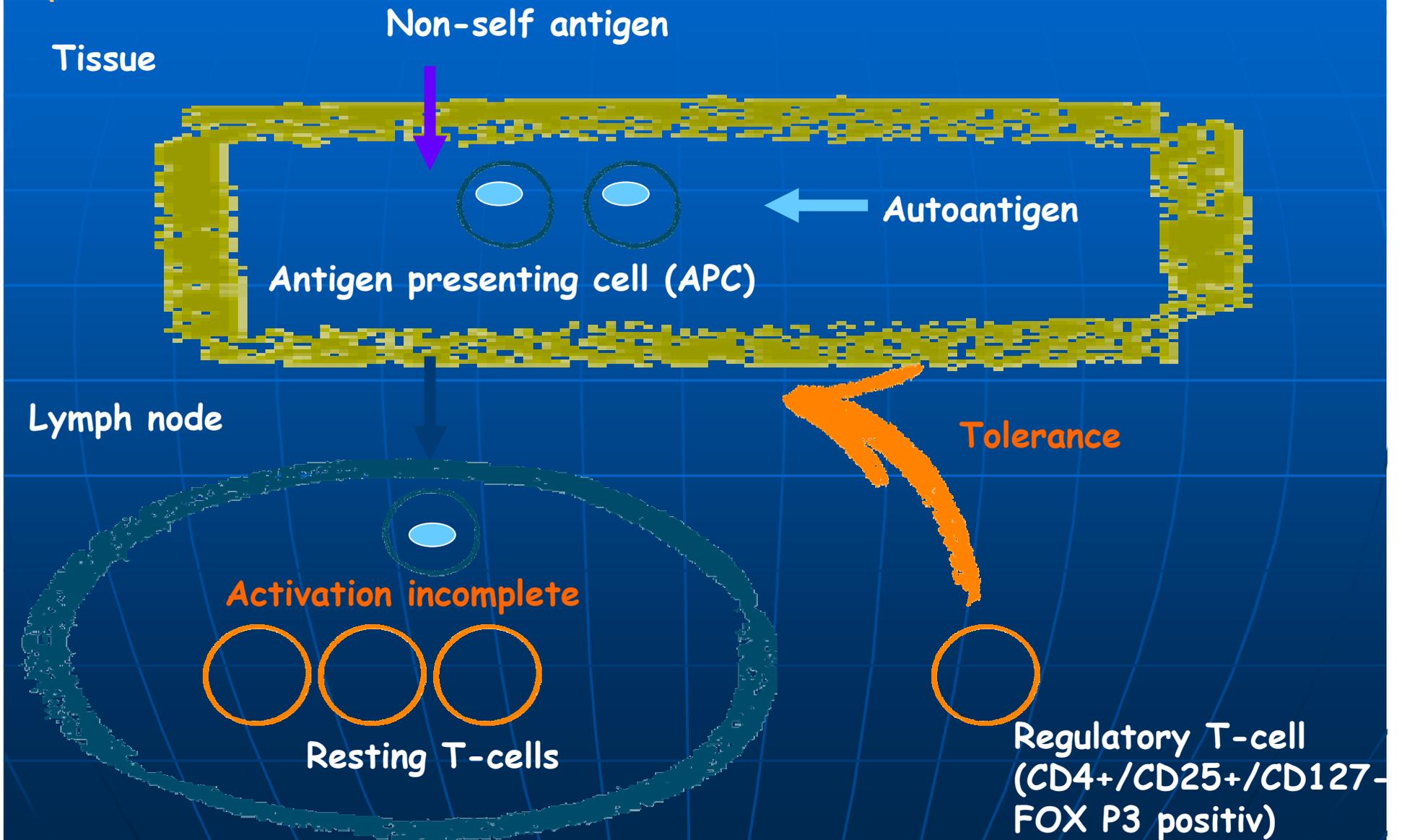
Principal of induction of a immunologic response



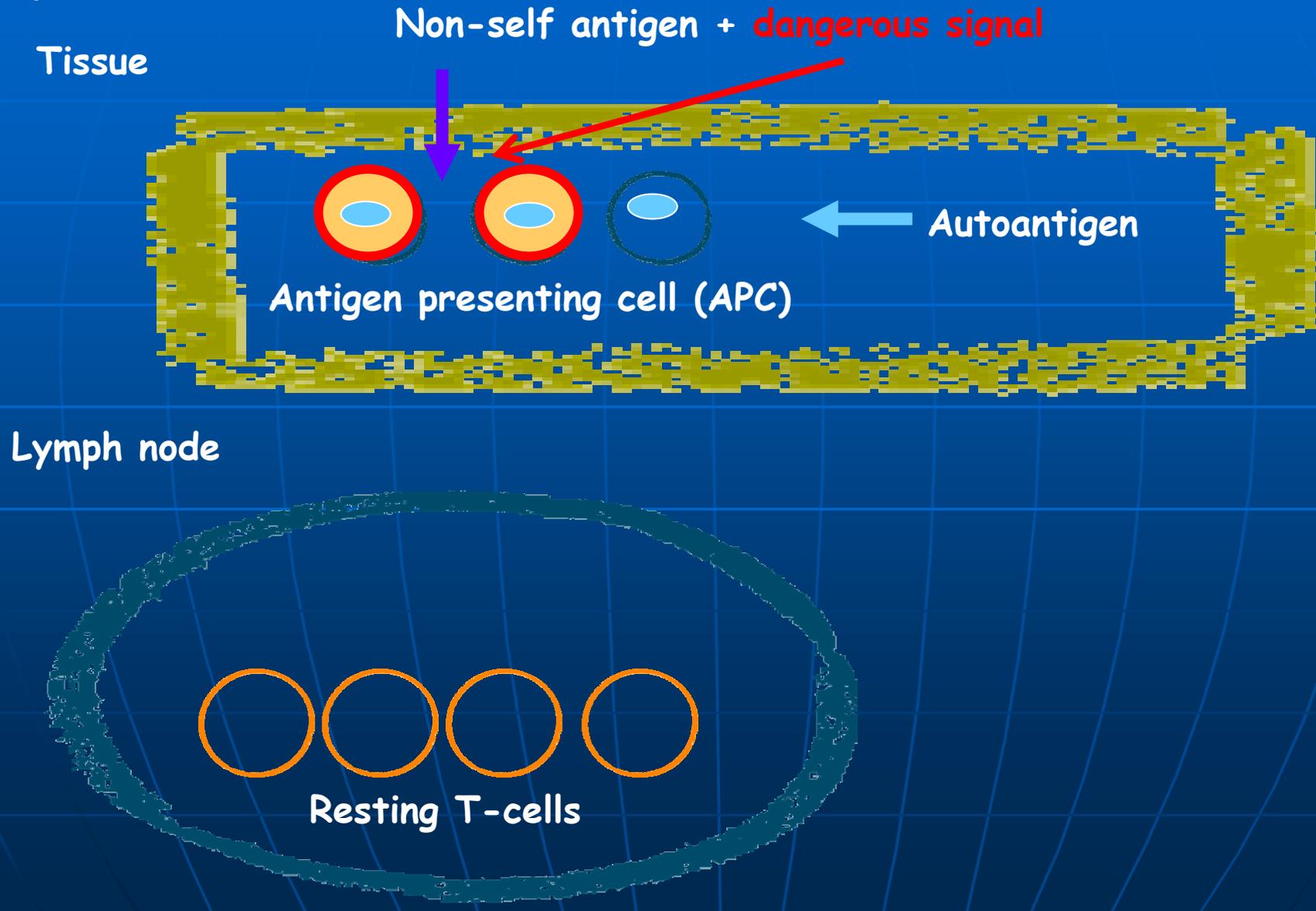
Principal of induction of a immunologic response



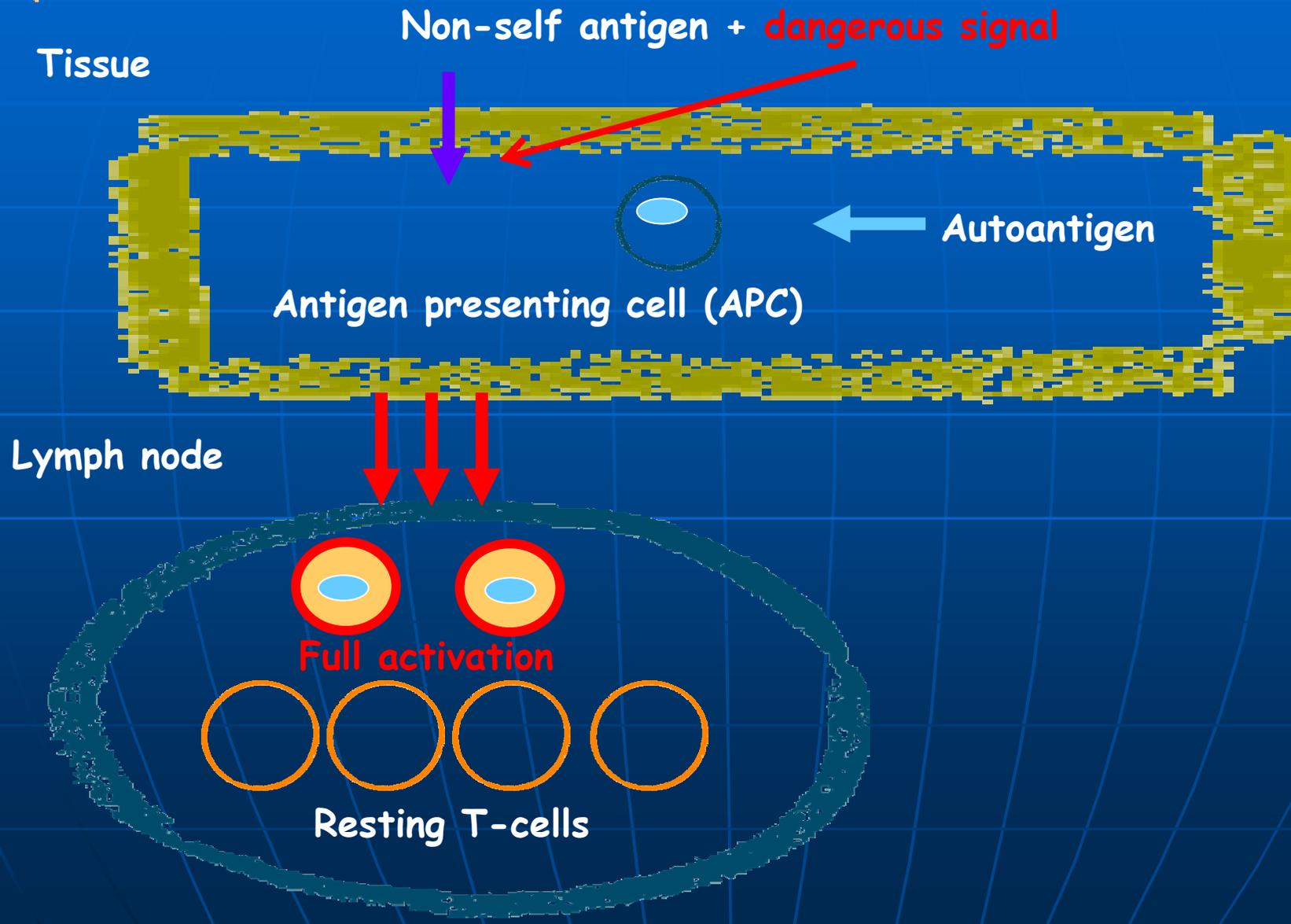
Not dangerous = Tolerance



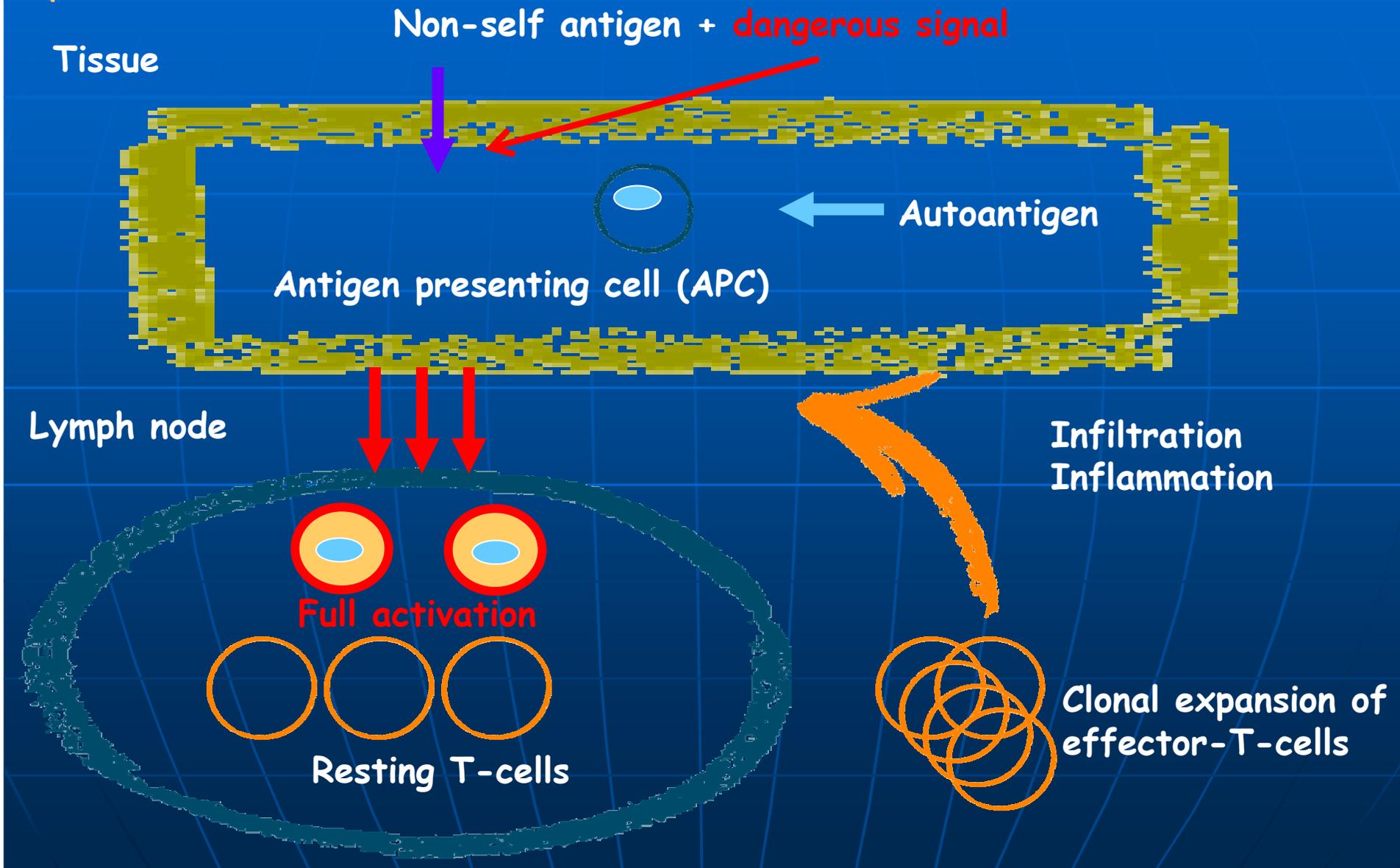
Principal of induction of a immunologic response



Principal of induction of a immunologic response



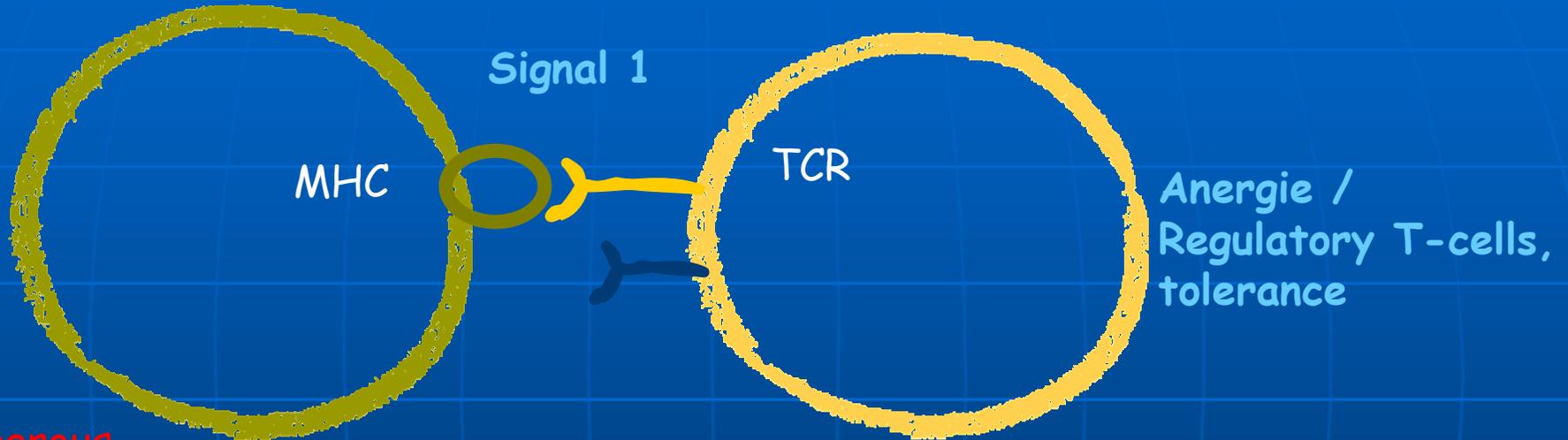
Dangerous = Activation of the immun system



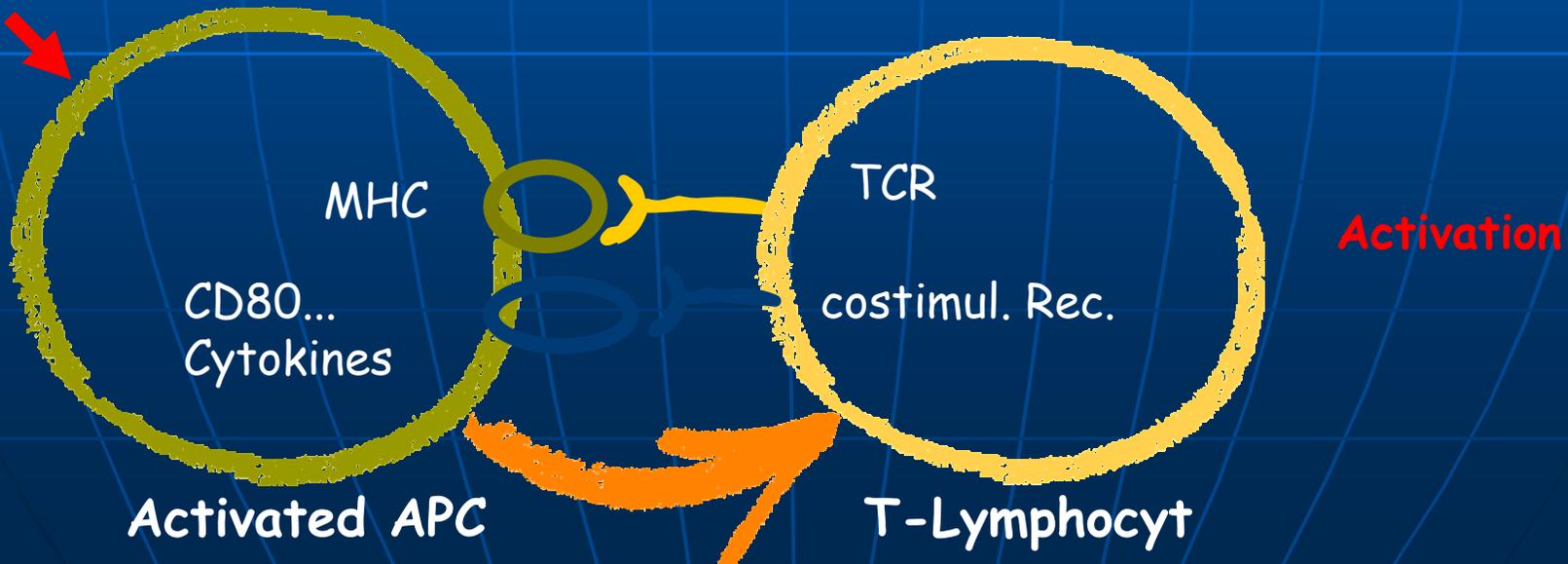
Activation of APC by „dangerous Signals“

resting APC

T-Lymphocyte



dangerous

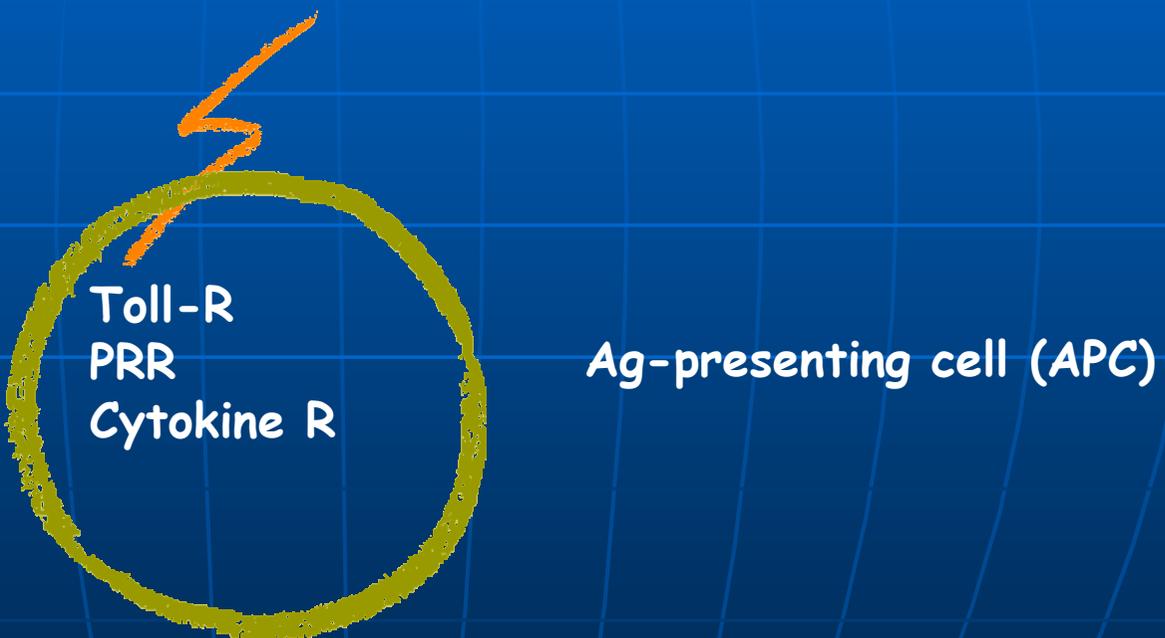


What are „dangerous“ signals ?

tissue injury
Hypoxia ...

Cytokines
TNF, IL-1, IL-2 ...

Microbial products
LPS, LTA, CpG DNA ...



Toll-R
PRR
Cytokine R

Ag-presenting cell (APC)

Transplantation: „surgeons are dangerous“
(ischemia/reperfusion)

„Classical“ therapeutical prevention of „dangerous signals“

Corticosteroid therapy	
Effect on	Physiological effects
↓ IL-1, TNF- α , GM-CSF ↓ IL-3, IL-4, IL-5, CXCL8	↓ Inflammation caused by cytokines
↓ NOS	↓ NO
↓ Phospholipase A ₂ ↓ Cyclooxygenase type 2 ↑ Annexin-1	↓ Prostaglandins ↓ Leukotrienes
↓ Adhesion molecules	Reduced emigration of leukocytes from vessels
↑ Endonucleases	Induction of apoptosis in lymphocytes and eosinophils

Steroids **inhibit**
NFkB activation

IL-2R-expression

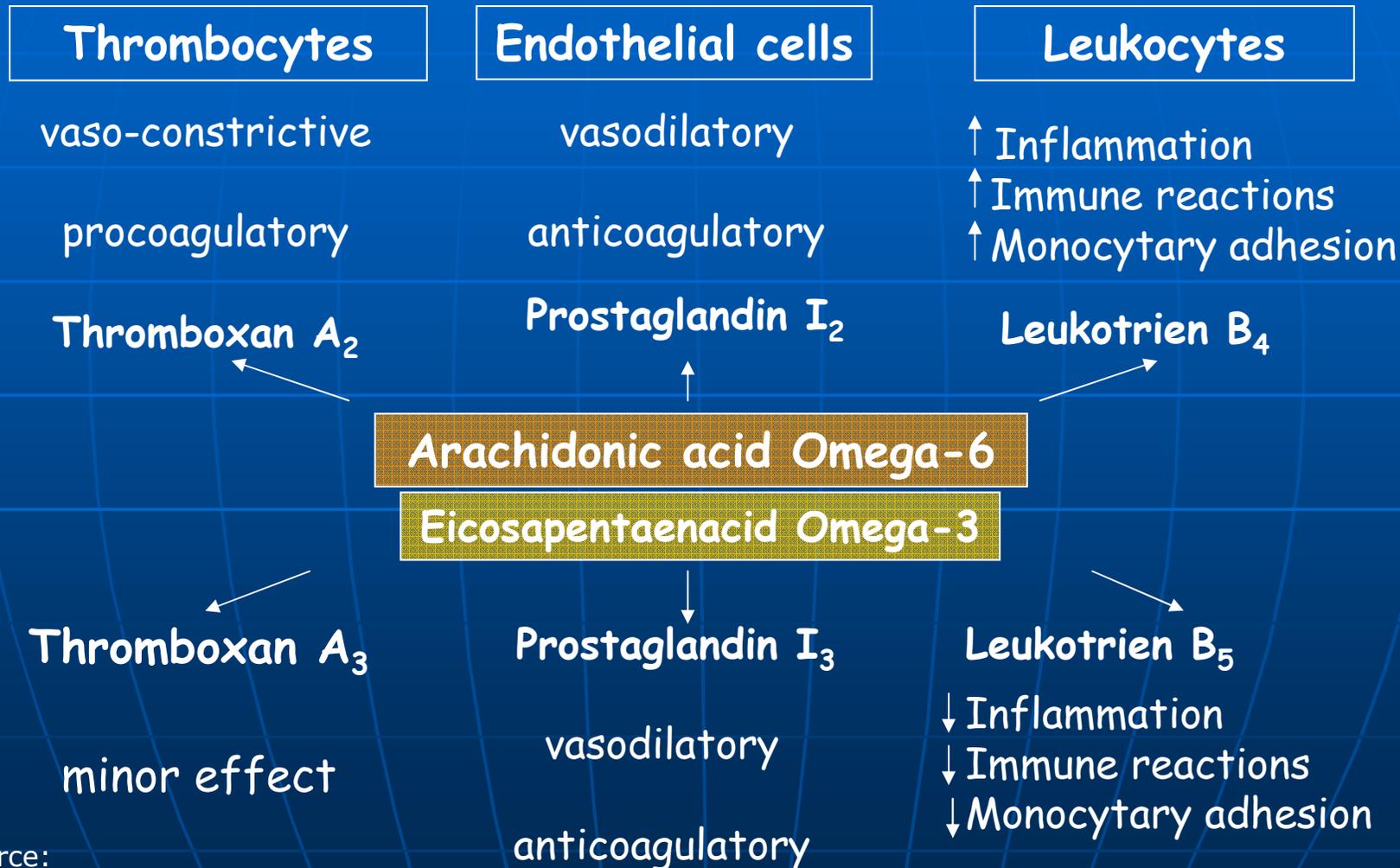
**CD4+-T-
 Cellproliferation**



The antiinflammatory potential of Omega-3-fatty acids from fish oil

- Ω Synthesis of Lipid mediators (Eicosanoids, Lipoxines, Resolvines, Protectines) with a modulating effect on:
 - Ω Blood clotting
 - Ω Blood pressure
 - Ω **Immune response**
 - Ω **Inflammation**
- Ω Integral part of biologic membranes
 - Ω Effect on membrane properties like viscosity
 - Ω Especially for DHA:
 - in brain and nerve tissue → Development of intelligence
 - in the retina → Visual functions
 - Ω Stabilization of heart rhythm

Different biologic effects of the Eicosanoids derived from Arachidonic acid (AA) and EPA



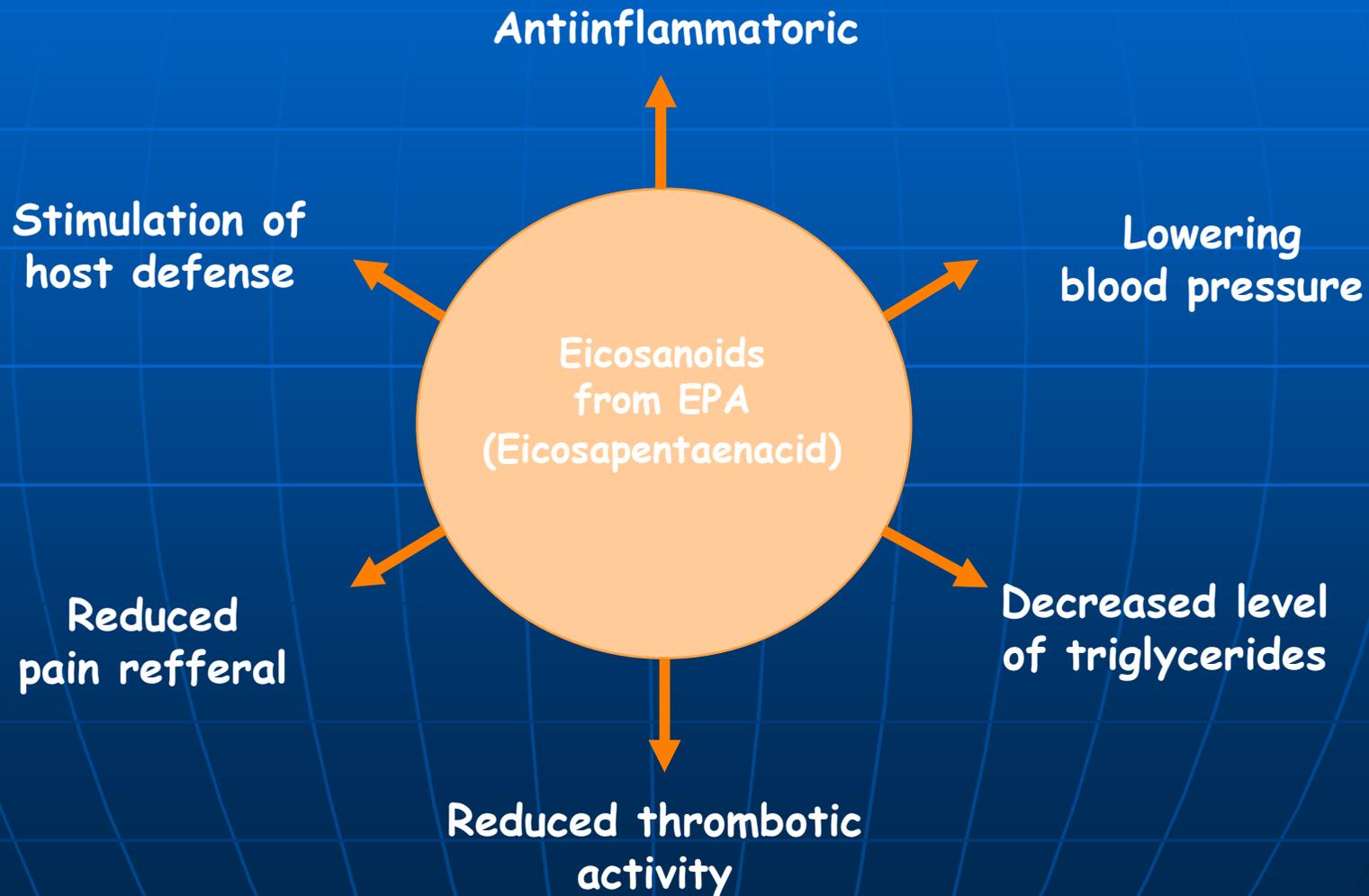
Source:

Trautwein, A. (1999): Fette und Fettbegleitstoffe. Omega-3-Fettsäuren. In: Erbersdobler, H. F., Meyer, A. H. (Hrsg.): Praxishandbuch Functional Food, Behrs Verlag Hamburg

Effects of Alimentation with less Arachidonic acid (AA) and more EPA

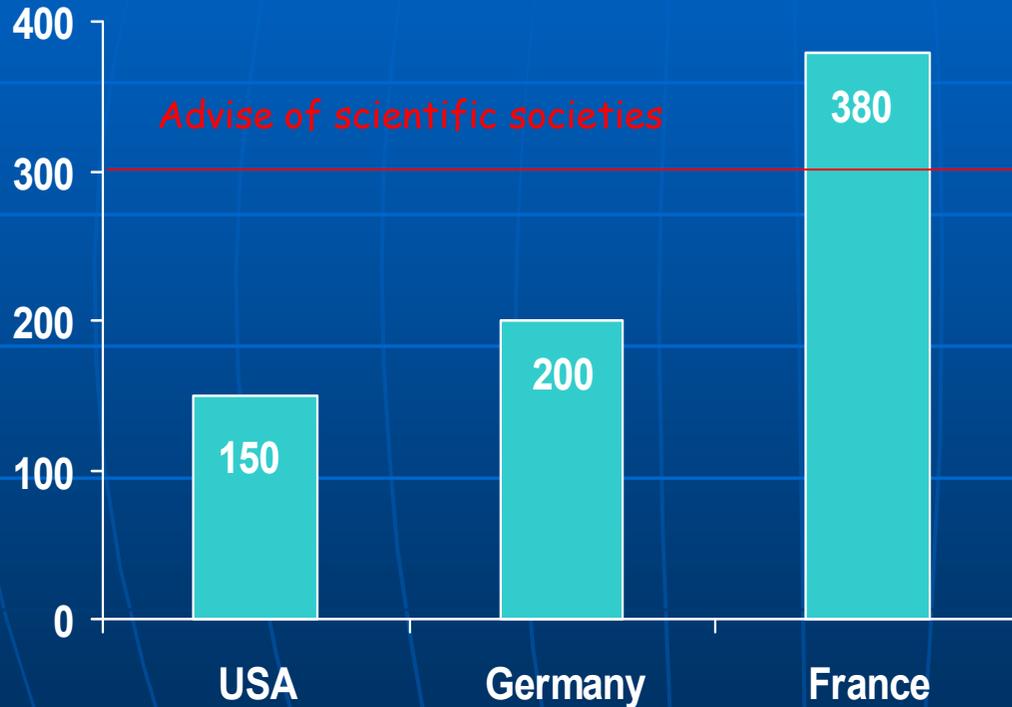
- Alimentation with less Arachadonic acids and more EPA leads to a reduced inflammatory potential by
 - Displacement of Arachidonic acid (AA) from cell membranes by EPA:
reduced synthesis of proinflammatory Eicosanoids and increased release of „healthy“ Eiconsanoids from EPA
 - reduced synthesis of the high proinflamma-toric Leukotrien B₄ (LTB₄) from Arachidonic acid

Positive Effects of Eicosanoids derived from EPA



The supply with Omega-3-fatty acids is insufficient

Administration of
EPA / DHA
in Milligramm



Average intake for adults per day

Source:

Ernährungsbericht der DGE (2004), Nationale Verzehrsstudie II (2008), Leipziger Lipid Studie (Richter; V. et al. (2007): Cardiovascular risk factor profile on a population basis: Results from the Lipid Study Leipzig. Exp Clin Cardiol; 12: 51-53)

Regulatory T-cells under Omega-3-supplementation - targets for optimizing the conditions in pregnancy ?

Evaluation of prognostic parameters for interventional tolerance induction by oral omega-3 fatty acid substitution during in vitro fertilization (IVF) therapy

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Objective: Aim of this prospective study was the evaluation of prognostic parameters for oral ω3-FA substitution in women undergoing in vitro-fertilization (IVF) therapy by flow cytometry. The thesis of this study can be seen in the assumption that oral ω3-substitution increases the frequency of successful IVF treatments by optimizing the immunological conditions for implantation.

Introduction

It has been reported that a large part of spontaneous abortions has no genetic origin. The data about the frequency of genetically motivated abortions vary [1], [2]. Some publications report that over 50% of the abortions have no genetic origin. In those cases haematological and immunological origins may be present [3]. Omega-3 fatty acids (ω3-FA) are known to have an immunomodulatory and antiinflammatory effect especially on the cellular immunosystem [4]. The regulatory T-cells (T_{reg}) as a part of the cellular immunosystem are the population for induction of tolerance in pregnancy [5]. This leads to the assumption that ω3-fatty acids might have a preventive effect on abortions with an immunological origin. The clientele of our survey were female patients of a fertility center as well as the Labor-Berndorf in Düsseldorf, Germany. With reference to the survey clientele which mainly recruits from Germany and the Netherlands it can be referred to the 2008's national consumption survey of the Max Rubner-Institute, in Germany which pointed out that all age groups show a too low ω3-fatty acid consumption. With reference to our own study it is of interest that normal consumption of ω3-fatty acids shows no severe side effects as the national consumption survey pointed out.

Methodology

79 females with a history of 2 or more miscarriages were included in this study. The follow-up included 23 patients undergoing IVF therapy. Blood was collected for analysis before and about 4 weeks after oral substitution of 800 mg/d salmon oil (Quassee Pharma, Germany), which is equal to 480 mg/d ω3-fatty acids. The following parameters were analyzed by flow cytometry using specific antibodies from Beckton Dickinson: Granulocytes, lymphocytes, CD4⁺T-helper cells, CD8⁺T-suppressor cells, CD56⁺NK cells and CD19⁺B-cells. T_{reg} cells, measured using antibodies against CD4, CD25 and CD127 from BeckmanCoulter were added to the panel later. SPSS was used for statistical analysis. For Median analysis the Wilcoxon-test was used.

In the course of this study the diagnostics shown in table 1 are carried out.

Table 1:

Sample 1	Sample 2
Addressess	
Taking of the blood sample	
Blood count	
Haemoglobin	Haemoglobin
Haematocrit	Haematocrit
MCV, MCH, MCHC	MCV, MCH, MCHC
Thrombocytes	Thrombocytes
Leukocytes	Leukocyte
Lymphocytes	Lymphocytes
Granulocytes	Granulocytes
Basophil granulocytes	Basophil granulocytes
Neutrophil granulocytes	Neutrophil granulocytes
Eosinophil granulocytes	Eosinophil granulocytes
Monocytes	Monocytes
Cellular immun status	
T cells (absolut)	T cells (absolut)
B cells (absolut)	B cells (absolut)
NK cells (absolut)	NK cells (absolut)
CD4 ⁺ T cells (absolut)	CD4 ⁺ T cells (absolut)
CD8 ⁺ T cells (absolut)	CD8 ⁺ T cells (absolut)
CD4 ⁺ CD25 ⁺ T _{reg} zellen	CD4 ⁺ CD25 ⁺ T _{reg} zellen

For immun status measurement the Beckton Dickinson® Multiset IMK Kit was used to stain T cells, B cells and NK cells. The T_{reg} cell staining carried out according to the staining pattern in table 2.

Table 2:

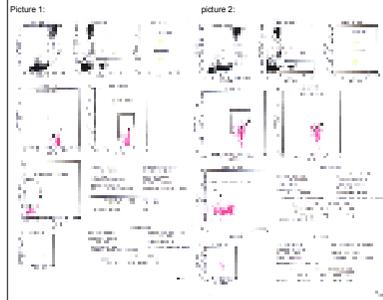
Sample (Minimum)	CD3	CD4	IFN1	IFN2
Sample (Maximum)	CD3	CD4	CD127	CD25

The control examinations were scheduled for atleast four weeks after beginning of the oral ω3-substitution therapy as shown in table 1.

1. Zhang, Y.X., et al., Genetic analysis of first-trimester miscarriages with a combination of cytogenetic karyotyping, microsatellite genotyping and array-CGH. Clin Genet. 2009, 78(2): p. 133-40.
2. Doria, S., et al., An efficient protocol for the detection of chromosomal abnormalities in spontaneous miscarriages or foetal deaths. Eur J Obstet Gynecol Reprod Biol. 2005, 147(2): p. 144-50.
3. Kwak-Kim, J., K.M. Yang, and A. Gilman Sachs, Recurrent pregnancy loss: a disease of inflammation and coagulation. J Obstet Gynaecol Res. 2009, 38(4): p. 609-22.
4. White, D., Dietary fatty acids. Am Fam Physician. 2009, 80(4): p. 345-50.
5. Zenzlén, A.C., et al., Immunology of pregnancy: cellular mechanisms allowing fetal survival within the maternal uterus. Expert Rev Mol Med. 2007, 9(10): p. 1-14.

Immunomodulation with Omega-3-fatty acids leads to reduced activation signs and an increase in the population of regulatory T-cells, optimizing the conditions for tolerating the fertilized egg

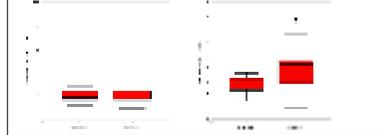
The pictures below are examples of our T_{reg} assay result screen. The picture on the left side (picture 1) shows a typical isotype control staining while the picture on the right (picture 2) shows a typical T_{reg} staining.



Results

The results obtained based on preliminary data since this study is still under process with more patients to be examined soon. As mentioned, we used the Wilcoxon tests to analyse the significance of the median values we obtained.

Our preliminary data shows a significant increase in the total CD3 positive lymphocyte population within the established reference value range (0,9-2,2 cells / nl) under oral ω3-intake (P < 0,001). This T-lymphocyte expansion is accompanied by a significant (p < 0,001) increase in the CD4⁺T cell population within the established reference range of 0,5-1,2 cells/ml. We further found a not significant increase in the relative frequency of CD4⁺CD25⁺T_{reg} cells represented in an increase in the relative amount of the CD4⁺CD25⁺Treg population among the CD4⁺T cell population (P: 0,0001). The picture below shows the box plots for the Treg population and the population of CD4⁺T-cells comparing values before and under oral intake of ω3-FAs after atleast four weeks as examples. Furthermore we found changes in the median value of granulocytes, B-lymphocytes and CD8⁺T-lymphocytes.



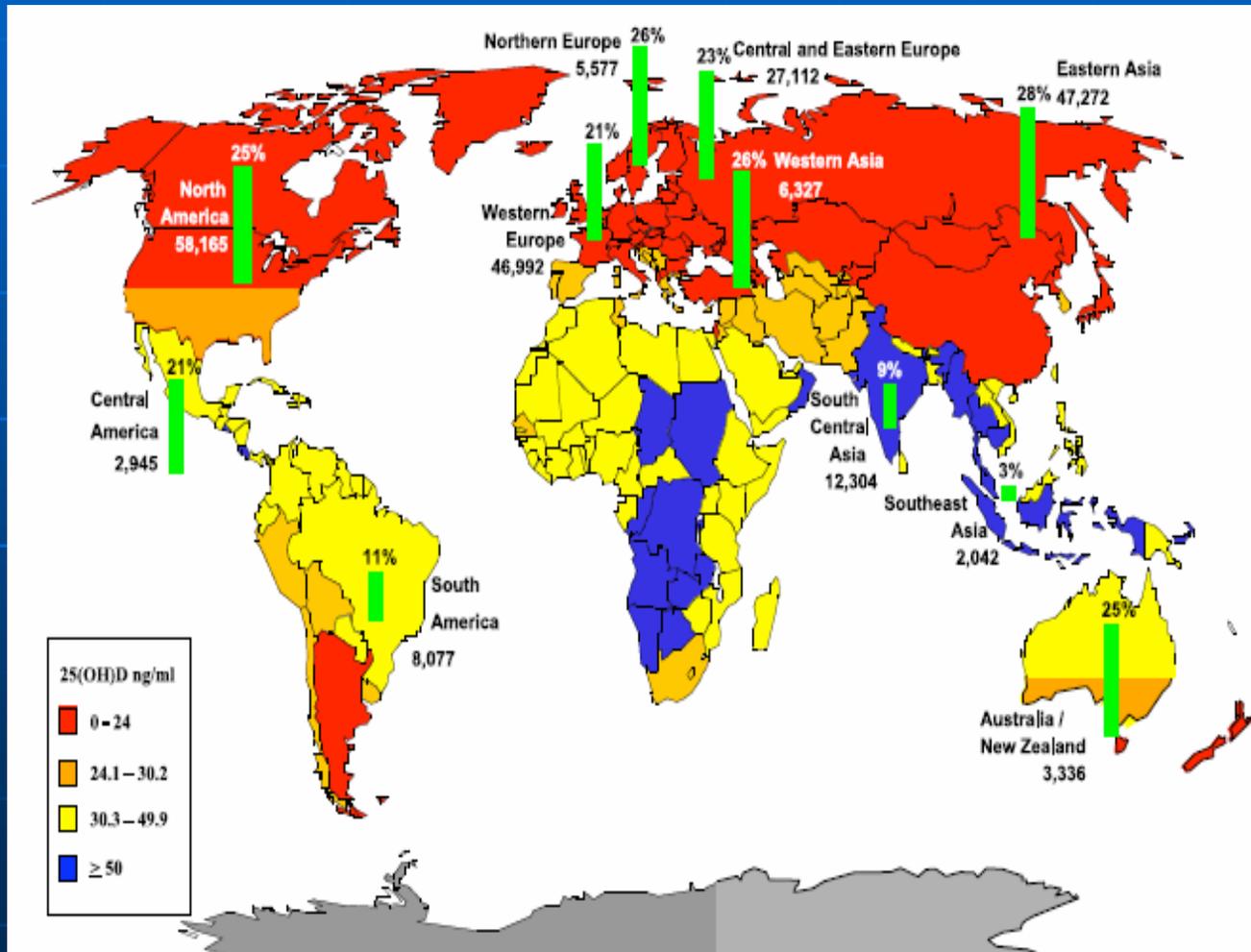
Conclusion

The flow cytometry analysis of parameters belonging to the cellular immun system, especially the CD4⁺T-lymphocytes and the regulatory T-cells, might be a useful tool for a laboratory monitoring of women undergoing a supportive immunomodulatory therapy with ω3-FAs in IVF-treatment. The oral intake of ω3-FAs leads to a significant expansion of CD4⁺T-lymphocytes within the established reference range which might partly be due to slight expansion of the CD4⁺CD25⁺Treg population. This increase is, though not significant, very interesting, since this population is considered the major contributor to maternal tolerance during pregnancy.

Summary

- The intake of Omega-3-fatty acids will improve the conditions for tolerating the fertilized egg by reducing „dangerous signals“
- Beside this immunologic effect, Omega-3-fatty acids have a positive effect in brain development of the fetus

Vitamin-D and fertility issues

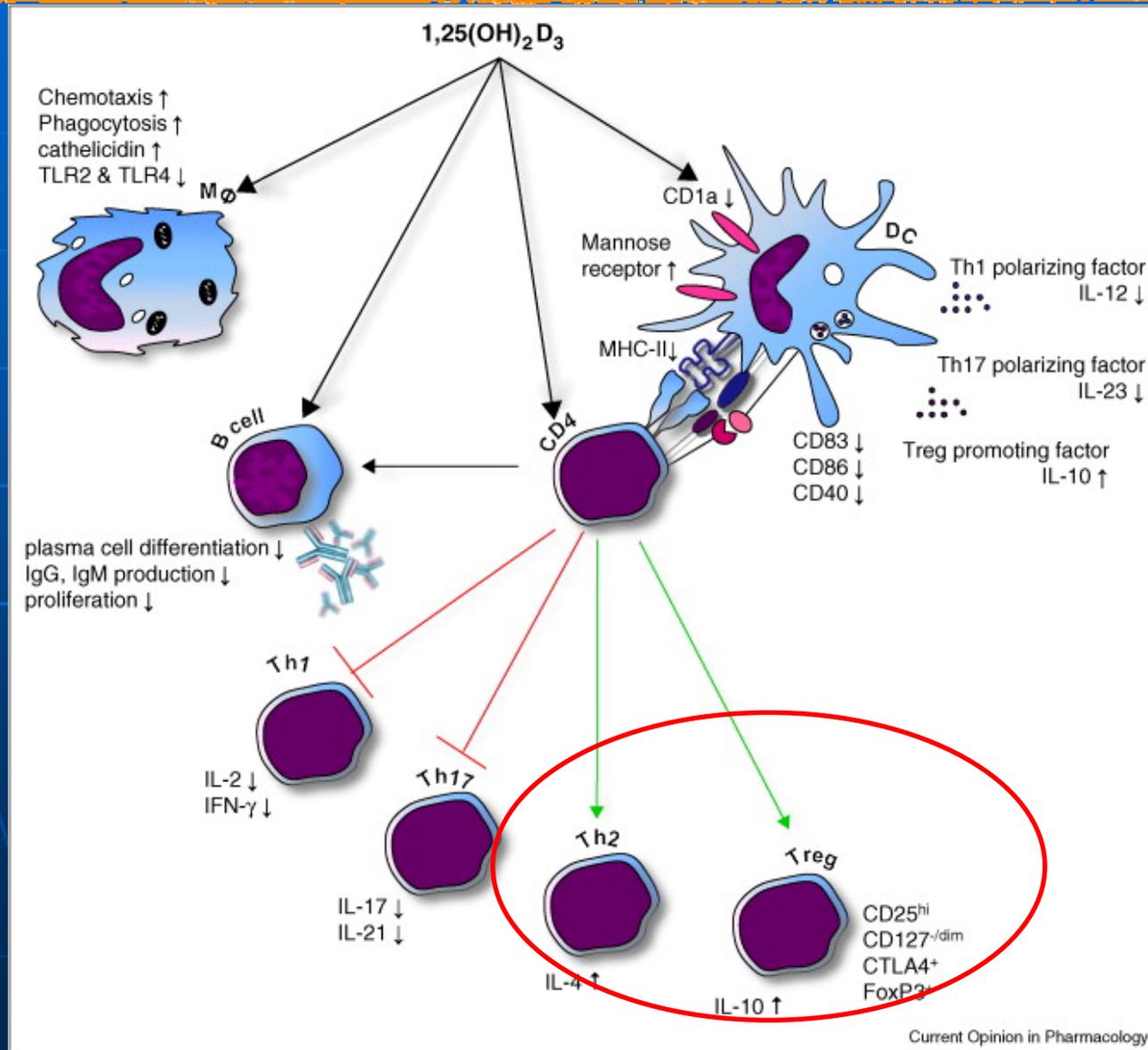


Interactive Map for Vitamin-D-publications regarding children/young adults and adults for each country worldwide

<http://www.iofbonehealth.org/facts-and-statistics/vitamin-d-studies-map>

Worldwide supply with Vitamin-D, published 2009

Vitamin-D and fertility issues



Vitamin-D and fertility issues

The immunoregulatory effect of Vitamin-D

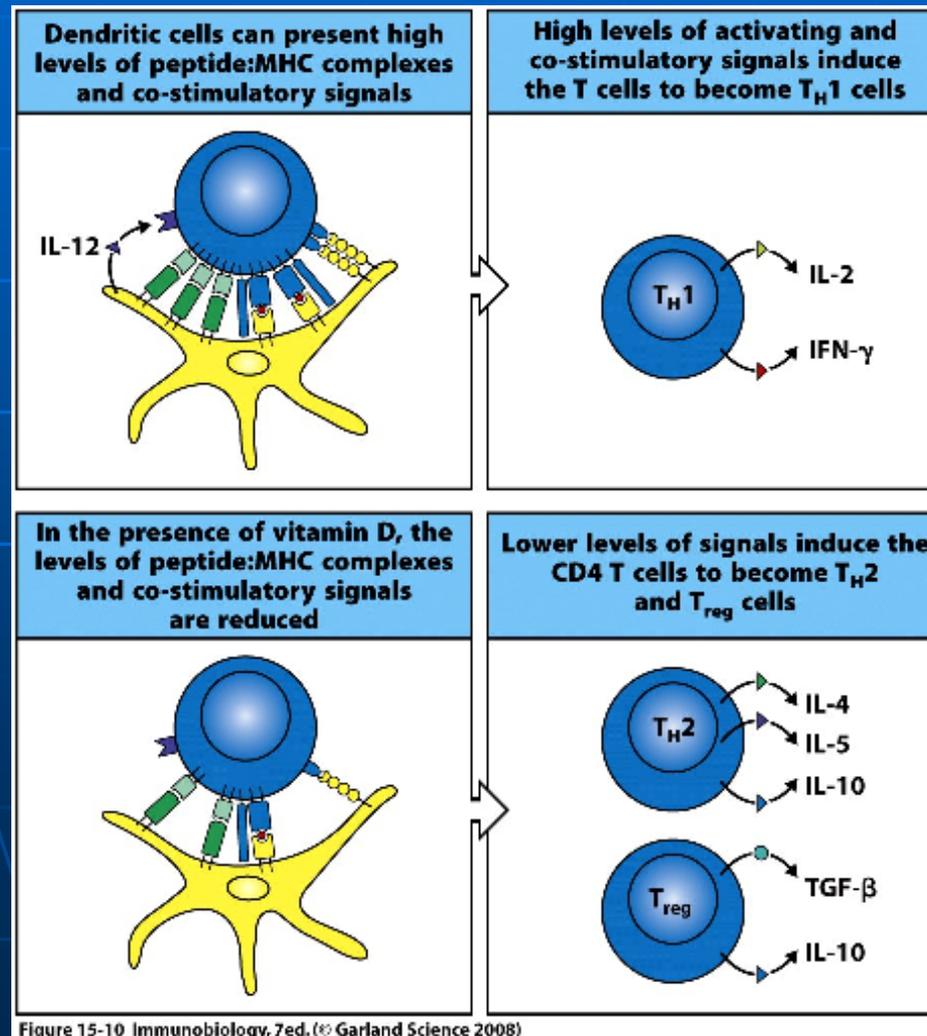


Figure 15-10 Immunobiology, 7ed. (© Garland Science 2008)

Summary

- Pregnancy fulfills the same criteria like a allotransplantation
- A specific cellular immune system with a good capability for inducing tolerance is one condition for a positive outcome in pregnancy
- Diseases accompanied by dangerous signals like proinflammation, autoimmune diseases, atopic disposition and infections will reduce consequently the ability for the induction of tolerance

Summary

- Screening for a lack of Vitamin-D and consequently substitution can increase the percentage of regulatory T-cells
- Base line laboratory tests, including T-cell-subsets, regulatory T-cells, pro- and antiinflammatory cytokines, could be a helpful tool in detecting immunologic disorders prior fertility treatment
- Monitoring regulatory T-cells during pregnancy might be a helpful prognostic tool

Summary

- Immunomodulation with Omega-3-fatty acids can optimize the conditions for nidation and pregnancy without significant side effects
- A additional therapy with steroids demands a strong indication, depending on the base line clinical and laboratory findings
- Due to the side effects of steroids, short term blood controls are strongly recommended

Thank you for your attention