Role of cytokines in Apitherapy

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Welcome to my Seminar
Cytokine Basics

- Cytokine or immunocytokine is a generic name used to describe a diverse group of soluble proteins and peptides which act as humoral regulators at nano- to- picomolar concentrations.

- Cytokines modulate the functional activities of individual cells and tissues both under normal and pathologic conditions.
What are cytokines?

- “Cytokines” are soluble protein secreted by the cells of innate and adaptive immunity and therefore mediate many of the functions of these cells.

- A subfamily of cytokines primarily functions in directing migration of cells, these are called “chemotactic cytokines” or “chemokines”.
General properties of cytokines

1. Most cytokines are low molecular weight polypeptides or glycoprotein (8~80 KD), and most of them are monomer.
General properties of cytokines

2. Natural cytokines are secreted by activated cells
   - Such as activated immune cells,
   - matrix cells
   - some tumor cells.
   - Ag,
   - SAg,
   - mitogen
3. One kind of cytokines can be produced by different cells. One kind of cells can secrete different cytokines.

General properties of cytokines

TH1
- IL-2
- IL-3, GM-CSF, TNF-α
- IFN-γ, TNF-β

TH2
- IL-4, 6
- IL-5
Cytokine Names

- **Interleukins** - produced exclusively by leukocytes
- **Lymphokines** - produced by lymphocytes
- **Monokines** - produced exclusively by monocytes
- **Interferons** - involved in antiviral responses
- **Colony Stimulating Factors** - support the growth of cells in semisolid medias
- **Chemokines** - promote chemotaxis.
Chemical Structure

- Low molecular weight proteins, <30kD
- High affinity for receptors
- Active in picomole amounts
Cytokines in the Immune Response

- **Innate immune response**
  - IL 1-(Macrophage)-fever, capillary effects
  - IL 6-(Macrophage)-adaptive immunity via B cells
  - IL 12(Macrophage)-adaptive immunity via T helper cells
  - TNF (Macrophage)-capillary effects, activates neutrophils
  - IFN *alpha* (Macrophage)-multiple effects
  - IFN *beta* (Fibroblasts)-multiple effects
Cytokines in the Immune Response

- **Adaptive immune response**
  - IL 2-(T cells)-multiple effects)
  - IL 4-(T cells & mast cells)-T cell differentiation, IgE production
  - TGF beta –(T cells, macrophages)-inhibits adaptive immune response
  - IFN gamma-(T cells, NK cells)-Macrophage activation
Cytokine Assays

- The biological activities of cytokines can be measured by a variety of bioassays which may employ factor-dependent cell lines, or antibodies (ELISA).

- RT-PCR quantitation of cytokines detects the presence of mRNA encoding specific cytokines.
WHAT IS APITHERAPY

“Apitherapy” is, simply said, the use of bee products to prevent, heal or recover somebody from one or more diseases/conditions.

The origin of this word is Api" comes from the bee's latin name: Apis mellifica

"therapy" comes from the Greek word "therapeuein" which means a method to treat the human beings or animals against different diseases.
WHAT IS APITHERAPY

- However, we understand today apitherapy in a much larger sense...

- Apitherapy is not just a simple, therapeutically method; it is already a different type of medicine.

- We can even call it "APIMEDICINE".
Honey Bees are beneficial insects

- Usually people think of bees for honey
Honey Bees are beneficial insects

- As pollinators—most valuable.
Honey Bees are beneficial insects

- Honey bees produce honey, wax, propolis, and royal jelly.
- Bee venom use for bee sting therapy (Apitherapy).
Honey Bees are beneficial insects

- Bee venom,
- Bee pollen,
- Honey,
- Royal jelly,
- Propolis

are products from bees that are generally considered to have medicinal effects (Hegazi, 2009 a,b and Hegazi, 2010).
Bee products

- Bee products, honey,
- royal jelly (RJ)
- bee pollen

belong to the extraordinaire components of human nutrition and are used in pharmaceutical and cosmetic industry.

These products contain physiologically active substances from floral origin of honey bee and plants origin (Aronne et al, 2014).
Bee products act upon both innate and adaptive immune response

- At different levels, in the human innate response, these compounds suppress DNA synthesis,
- decrease proinflammatory cytokine synthesis (IL-2, IL-12 and IL-4),
- inactivate both the classical and alternative complement pathway,
- decrease superoxide anion production in rabbit neutrophils.
In adaptive immune response

- propolis and honey induce the increase of antibody production by plasma cells,
- enhance the secretion of TGF-β after the activation of T regulatory cells,
- inhibit Con A-stimulated cell proliferation in mice (Cova, 2013).
In adaptive immune response

The effect of IL-10+ NK cells on Ag-specific T cell proliferation has been examined in bee venom major allergen, phospholipase A2- and purified protein derivative of Mycobacterium bovis-induced T cell proliferation.
In adaptive immune response

- IL-10+ NK cells significantly suppressed both allergen/Ag-induced T cell proliferation and secretion of IL-13 and IFN-gamma, particularly due to secreted IL-10 as demonstrated by blocking of the IL-10 receptor.
- These results demonstrate that a distinct small fraction of NK cells display regulatory functions in humans.
Honey

- Honey has been used since ancient times as a remedy in wound care and antimicrobial activity (Hegazi et al., 2015)
- Certain inflammatory cytokines, particularly IL-1β and TNF-α, can also induce the production of the keratinocyte growth factor which can indirectly promote re-epithelialization (Kristensen et al., 1993 and Brauchle et al. 1994).
Keratinocytes express relatively high amounts of matrix metalloproteinase-9 (MMP-9), and this production can be additionally up-regulated by TGF-β, TNF-a or IL-1β (Salo et al. 1994).
Honey

Keratinocytes, which are known to be involved in wound healing, are responsible for elevated production of mediators including cytokines (TNF-α, IL-1β and TGF-β) and matrix metalloproteinase-9 (MMP-9) after incubation with honey (Majtan et al. 2010).
The roles of tumor necrosis factor-a (TNF-a), interleukin-1b (IL-1b) and interleukin-6 (IL-6) are also important in the inflammatory response.
Honey

- Important role for natural honey in modulating immune response.
- **Tonks** et al., (2003) investigated the effects of honey on the activation state of immunocompetent cells, using the monocytic cell line, MonoMac-6 (MM6), as a model for the release of important inflammatory cytokines from MM6 cells.
Honey

- They assayed the tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β and IL-6.

- All honeys significantly increased the TNF-α, IL-1β and IL-6 release from MM6 cells (and human monocytes) when compared with untreated and artificial-honey-treated cells ($P<0.001$).
Honey

The production of pro-inflammatory cytokines IL-1β and TNF-α by human monocytic cell line in the presence of honey proteins was analyzed.

Honey proteins did not affect the production of IL-1β; however, TNF-α production was significantly suppressed (Mesaik et al., 2015).
Honey

- Tonks et al. (2003) suggested that the wound healing effect of honey may in part be related to the release of inflammatory cytokines from surrounding tissue cells, mainly monocytes and macrophages.

- The findings show that natural honeys can induce IL-6, IL-1β, and TNF-α release.
Honey

Honey has been shown to have mitogenic activity on human B and T lymphocytes.

The proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentrations as low as 0.1%; and phagocytes are activated by honey at concentrations as low as 0.1% (Abuharfeil et al., 1999).
Honey (at a concentration of 1%) also stimulates monocytes in cell culture to release cytokines, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, IL-6, which activate the immune response to infection (Tonks et al., 2001)
Honey

- Proteins present in honey will be highly glycosylated because of high sugar content.

- Glycosylated proteins have been shown to activate a number of cell types including monocyte cells (Brownlee, 1995).
Royal jelly
Royal jelly

- The protein fraction of RJ contains many valuable components and biologically active substances.

- Besides the MRJPs, low amounts of several minor proteins including antibiotics peptides (Fujiwara et al., 1990; Bilikova et al., 2001; Bilikova et al., 2002) are present in RJ.
Royal jelly

- Kohno et al., (2004) examined the anti-inflammatory actions of royal jelly (RJ) at a cytokine level.

- When supernatants of RJ suspensions were added to a culture of mouse peritoneal macrophages stimulated with lipopolysaccharide and IFN-gamma, the production of proinflammatory cytokines, such as TNF-alpha, IL-6, and IL-1, was efficiently inhibited in a dose-dependent manner without having cytotoxic effects on macrophages.
Royal jelly

- This suggests that **RJ contains factor(s) responsible for the suppression of proinflammatory cytokine secretion.**
- They named the factor for honeybee’s RJ-derived anti-inflammatory factor (HBRJ-AIF)
- **Royal jelly treatment in lymphocytes from patients with Graves' disease shifted the Th1/Th2 cytokine ratio to the side of Th1 cytokine.**
Pollen
Pollen

- At the mucosal surfaces, pollen grains do not only release allergens but also proinflammatory and immunomodulatory lipids, termed pollen-associated lipid mediators.
- Among these, the E(1)-phytoprostanes (PPE(1)) were identified to modulate dendritic cell (DC) function:
Pollen

- PPE(1) inhibit the DC's capacity to produce IL-12 and enhance DC mediated T(H)2 polarization of naive T cells.
Pollen-derived PPE(1) modulate DC function which lead to inhibition of NF kappa B activation and result in reduced DC IL-12 production and consecutive T(H)2 polarization.
Allergic asthma results from inappropriate T(H)2-mediated inflammation.

Both IL-4 and IL-13 contribute to asthma pathogenesis,

but IL-4 predominantly drives T (H) 2 induction,

whereas IL-13 is necessary and sufficient for allergen-induced airway hyper responsiveness and goblet cell hyperplasia.
Propolis

- Essentially the “glue” in bee hives.
- Made of plant resin.
- Preserves warmth in hive and keeps out microbes.
- Has various antimicrobial properties.
- Used for healing and part of “apitherapy”.
- Interesting uses including violin varnish.
- Used since the Ancient Egyptian, Greeks and Romans.
Propolis in the Hive

(Kulincevica & Gacica, 1991)
Propolis Major Components

- Caffeic acid phenethyl ether or CAPE.
- Phenolics
- Terpenes
- Hydrocarbons
- Acids
- Flavonoids
Properties of Propolis in General

- Stimulates antibody production.
- Inhibits viral entry into CD4 lymphocytes, especially against HIV-1.
- Increases effectiveness of antiviral drugs such as the reverse transcriptase inhibitor, zidovudine.
Properties of Propolis in General

- Treats opportunistic infections that plague AIDS patients.
- Decreases lymphocyte proliferation when exposed to mitogens such as ConA.
- Increases production of IFN-γ and activates macrophages.
Interesting Properties of CAPE

- Inhibits Nuclear Transcription Factor Kappa B or NF-κB, which drives T-cell proliferation and effector functions.
- Anti-inflammatory activity.
- Treats arthritis and inflammatory bowel disease.
- Inhibits IL-2 which also drives T-cell proliferation.
**Propolis** Increased Antibody Production

- Propolis was shown to increase antibody production in rats immunized with bovine serum albumin.
- Acted as adjuvant.
- Enhanced the activity of macrophages.
Propolis Inhibition of NF-κB

- CAPE inhibited NF-κB binding to macrophages and decreased cytokine production.
- Tumor necrosis factor alpha, TNF-α, which stimulates macrophages to kill tumor cells was used to see if NF-κB would bind.
- Anti-inflammatory activity.
- Macrophages underwent apoptosis in patients with IBD leading to healing of the injuries to the colon.
Propolis Inhibition of NF-κB

(Fitzpatrick, Wang, & Le, 2001)
CAPE Induces Apoptosis in Macrophages in Patients with IBD

(Fitzpatrick, Wang, & Le, 2001)
CAPE Reduces Injury to the Colon

(Fitzpatrick, Wang, & Le, 2001)
Propolis Inhibition of IL-2

- CAPE inhibited IL-2 leading to anti-inflammatory activity.
- T-cell proliferation was inhibited in samples with Con-A, a mitogen, added.
Propolis Anti-viral Activity

- Viral entry of HIV-1 was inhibited in CD4 lymphocytes.
- Effectiveness of the reverse transcriptase inhibitor, zidovudine, was increased.
- Virus was kept from proliferating.
Propolis Decreased Viral Expression in CD4 Cells

(Gekker, Hu, Spivak, Lokensgard, & Peterson, 2005)
Propolis Increases Effectiveness of Anti-viral Drugs

(Gekker et. al, 2005)
Propolis Treatment for AIDS Patients

- Propolis treats opportunistic fungal infections such as thrush and leukoplakia.
- Kept infections from coming back and alleviated symptoms.
- Increased the immune response.
Propolis Increased Production of IFN-γ

- Propolis increased IFN-γ production leading to the antigen being presented on cells and the immune response starting to clear it faster.
- Mitogen infected cells did not show proliferation that would normally happen.
- Kept mitogen from working.
Summary

- Propolis is an effective anti-inflammatory agent.
- Can be used to help AIDS patients.
- Controls inflammatory diseases.
- Increases effectiveness of immune system.
- Mechanisms are not known yet.
- Ancient cure for today’s ailments.
Propolis

Propolis, the resinous product collected by honey bees from plants, is used as folk medicine since ancient time.

During the last ten years, immunoregulatory and anti-inflammatory properties of propolis have been published.

Inflammatory cytokines and oxidative stress have a central role in the pathogenesis of acute pancreatitis.

Propolis has anti-inflammatory and anti-oxidant effects.
Propolis

Song et al., (2008) found that the anti-inflammatory effect of caffeic acid phenethyl ester (CAPE) is due to its inhibition of tumor necrosis factor (TNF)-alpha expression and interleukin (IL)-8 production.
Propolis

The anti-inflammatory effect of CAPE is possibly through the inhibition of nuclear factor (NF)-kappa B via the suppression of inhibitor-kappa B-alpha (IkappaB-alpha) degradation
Choi and Choi (2008) reported that (i) CAPE exerts its anti-inflammatory action (inhibition of tumor necrosis factor-induced expression of intercellular adhesion molecule-1 and CC chemokine ligand-2) via NF-kappa B inhibition by two distinct molecular mechanisms in a cell-specific manner:
Propolis

- CAPE inhibited downstream pathways of inhibitor kappaB (IkappaB) degradation in monocytic cells, while activation of upstream IkappaB kinase was suppressed by CAPE pre-treatment in astroglial cells.

- and (ii) CAPE paradoxically activates the c-Jun N-terminal kinase (JNK) pathway, which might be responsible for its pro-apoptotic action and divergent regulation of proinflammatory mediators such as CXC chemokine ligand-8.
Propolis

- Sy et al., (2006) found that the higher dose of propolis extracts decreases the level of IL-5 in BALF.

- The splenocytes from mice administered with propolis extracts (low- and high-dose groups) exhibit a strong inhibition of IL-10 secretion and up-regulation of IFN-gamma secretion in splenocytes stimulated with concanavalin A (ConA).
Propolis

- Propolis stimulated splenocytes
- Cytokine (IFN-gamma, IL-6, and IL-10) secretion
- These results suggest that propolis extracts may be a potential novel therapeutic agent for asthma.
Propolis

- CAPE suppressed H. pylori-induced cell proliferation and production of the cytokines TNF-alpha and IL-8.
- In addition, CAPE blocked H. pylori-induced COX-2 expression.
- The inhibition of such transcription by CAPE could result in suppression of many genes during H. pylori-induced inflammation (Abdel-Latif et al., 2005).
Propolis

Márquez et al., (2004) found that CAPE specifically inhibited both interleukin (IL)-2 gene transcription and IL-2 synthesis in stimulated T-cells.
Propolis

- Takagi et al., (2005) found that cytokines released from macrophages in mouse peripheral blood after Propolis administration activated helper T-cells to proliferate.
- Activated macrophages in association with the secondary T-lymphocyte activation increased IFN-gamma production and stimulated proliferation of cytotoxic T-cells and suppressor T-cells, indicating the activation of cell-mediated immune responses.
Propolis

- Blonska et al., (2004) indicated that EEP exerts its inhibitory effect on the IL-1beta and iNOS gene expression in J774A.1 macrophages at the transcriptional level.
- Tested flavone derivatives contribute to the anti-inflammatory activity of propolis.
- The cytokine, IL-2, IL-4 and IFN-gamma were significantly increased at the dose of 20 mg/kg CAPE group.
- These results suggest that CAPE could have immunomodulatory effects in vivo (Park et al., 2004).
Bee venom (BV)
Bee venom (BV)

- Bee venom contains a number of powerful anti-inflammatory substances, including adolapin and melittin.
- It is to be a hundred times more powerful than hydrocortisone,
- melittin stimulates the body production of cortisol, a natural steroid that also acts as an anti-inflammatory.
In adaptive immune response

- Its effect of IL-10+ NK cells on Ag-specific T cell proliferation has been examined in bee venom major allergen, phospholipase A2- and purified protein derivative of *Mycobacterium bovis*-induced T cell proliferation.
Bee venom (BV)

- Moon et al., (2007) demonstrate that BV and MEL possess a potent suppressive effect on proinflammatory responses of BV2 microglia.

- They suggested that these compounds may offer substantial therapeutic potential for treatment of neurodegenerative diseases that are accompanied by microglial activation.
Bee venom (BV)

- Mellitin had no effect on IL-1beta- or TNF-alpha-induced MMP1 or MMP3 production and did not decrease LPS-induced secretion of MMP1.
- Among the serum proinflammatory cytokines, the production of TNF-alpha in the BV group was suppressed compared to the control group but IL-1beta was not suppressed.
Bee venom (BV)

In vivo bee venom treatment affects the production of IL-1 by macrophages directly (Hadjipetrou-Kourounakis and Yiangou, 1988).
Bee venom (BV)

- Korean bee venom (KBV) has anti-inflammatory properties that inhibit NOS and TNF-α expression.
- KBV could be useful in inhibiting the production of inflammatory cytokine and NO production in neurodegenerative diseases (Han et al., 2007).
Hegazi et al., (2013) found that Propolis and bee venom are effective in treatment of psoriasis, with minimal tolerable side effects, when used either separately or in combination. A significant reduction in both PASI score and serum level of IL-1β was observed in all groups of patients.
Bee venom (BV)

Correlation between percentage reduction of PASI score and that of IL-1 \( \beta \) showed a strong positive correlation in group I received bee venom.
Continuous exposure of non-allergic beekeepers to high doses of bee venom antigens induces diminished T cell-related cutaneous late-phase swelling to bee stings in parallel with suppressed allergen-specific T cell proliferation and T helper type 1 (Th1) and Th2 cytokine secretion.
Bee venom (BV)

- Meiler et al., (2008) found after multiple bee stings, venom antigen-specific Th1 and Th2 cells show a switch toward interleukin (IL) 10-secreting type 1 T regulatory (Tr1) cells.
- T cell regulation continues as long as antigen exposure persists and returns to initial levels within 2 to 3 mo after bee stings.
- Histamine receptor 2 up-regulated on specific Th2 cells displays a dual effect by directly suppressing allergen-stimulated T cells and increasing IL-10 production.
Bee venom (BV)

- Kim et al., (2008) found that bee venom, injected i.p at doses of more than 20 microl/100g mouse once a day for 14 days inhibited the ability of inguinal lymph node cells to produce:
  - T cell cytokines interleukin-1beta, -2, -6,
  - tumor necrosis factor-alpha
  - and interferon-gamma.
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