Role of Bioactive Plant Polysaccharides for Health Care

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Problems in Health Care in Modern Times

- increasing endogenously caused chronic disorders
- increasing non-specific, constitutional or psychosomatic diseases
- increasing population of aged persons

Even today, 75% of the world population still relies on plant, plant extracts and other tools of traditional medicine to assist in basic health care (WHO).

Medicinal Plants

- Plant derived
- Functional Foods

Low molecular weight ingredients

- Macromolecules
  - polysaccharides (content: 5-10%)
  - proteins
  - lignins/lignin-carbohydrate complexes
  - Tannins

Pharmacological actions

Clinical Efficacy

Health care

Attract worldwide attention to traditional herbal medicines and functional foods as an alternative treatment and prevention.
### Pharmacological activities of plant polysaccharides

#### Immune/hemopoietic system (Potentiation)
- ★ Antibody production enhancing activity (including vaccine adjuvant activity)
- ★ Host-mediated anti-tumor activity
- ★ Anti-infectious activity (*Staphylococcus pneumoniae/ Cytomegalovirus/ Influenza virus infections*)
- ★ Improvement of erythropoietin-resistant anemia

#### Digestive system
- ★ Anti-emetic activity
- ★ Inhibition of absorption of heavy metal
- ★ Anti-ulcer activity
- ★ Prevention of colon cancer (Induction of apoptosis, SCFA-dependent)
- ★ Improvement of blood glucose level on diabetes model mice

#### (Suppression)
- ★ Anti-inflammatory activity
- ★ Suppression of metastasis of prostate cancer/lung cancer
- ★ Improvement of mucositis
- ★ Improvement of chronic renal disorder
- ★ Improvement of LPS-induced sickness behavior

#### Other
- ★ Enhancement of Long-term potentiation on central nervous system (cytokine-dependent)
- ★ Lowering effect of blood cholesterol level (Induction of drug transporter/drug metabolizing enzyme)
Structure of Pectic Polysaccharides (J. P. Vincken et al., 2003)

**Pectin**

- **rhamnogalacturonan I (RGI)**
  - $-2\alpha\text{Rha} - 4\alpha\text{GalA} - 2\alpha\text{Rha} - 4\alpha\text{GalA} - 2\alpha\text{Rha} - 4\alpha\text{GalA} - 2\alpha\text{Rha} - 4\alpha\text{GalA}$
  - $\beta\text{Gal}$

- **rhamnogalacturonan II (RGII)**
  - $\alpha\text{GalA} - 2\beta\text{Rha} - 4\beta\text{Gal}$
  - $2\alpha\text{MeXyl} - 3\alpha\text{Fuc}$
  - $\beta\text{GlcA}$
  - $\alpha\text{Gal}$

- **homogalacturonan (HG)**
  - $-4\alpha\text{MeGalA} - 4\alpha\text{GalA}$
  - $\beta\text{Gal}$

- **xylogalacturonan (XG)**
  - $-4\alpha\text{GalA} - 4\alpha\text{GalA}$
  - $\beta\text{Api}$
  - $\alpha\text{Rha}$
  - $3\beta\text{AceAf}$
  - $\text{AcO} - 2\alpha\text{MeFuc}$
  - $\beta\text{Araf}$

- **arabinogalactan I (AG-I)**
  - $-4\beta\text{Gal} - 4\beta\text{Gal} - 4\beta\text{Gal} - 4\beta\text{Gal} - \alpha\text{Araf}$

- **arabinan**
  - $-\alpha\text{Araf} - 5\alpha\text{Araf} - 5\alpha\text{Araf} - 5\alpha\text{Araf} - 5\alpha\text{Araf} - 5\alpha\text{Araf} - 5\alpha\text{Araf}$

- **arabinogalactan II (AG-II)**
  - $-3\beta\text{Gal} - 3\beta\text{Gal} - 3\beta\text{Gal} - 3\beta\text{Gal} - 3\beta\text{Gal} - 3\beta\text{Gal}$
  - $\beta\text{Gal}$
  - $\alpha\text{Araf}$
  - $\beta\text{Araf}$
How do polysaccharides affect physiological systems through intestine?

Peyer’s patch (100-200/human)

ILF (30,000/human)

oral administration

Polysaccharides

Gut-associated lymphoid tissue (GALT)
- Peyer’s patches
- Isolated lymphoid follicles (ILF)
- Intraepithelial lymphocytes (IEL)
- Lamina propria lymphocytes (LPL)

Physiological systems
- Immune
- Central/peripheral nervous
- Endocrine
- Hemopoietic etc.

Epithelial cells

M cells

Peyer’s patch

Direct absorption

Intestine
Plant polysaccharides
Pectic polysaccharides
Starch
Cellulose

Intestine (Jejunum, Ileum)
- Microflora \((10^3 - 10^7 \text{ organisms/g})\)
- High yield of polysaccharides are recovered
- Incorporation into lymphoid follicle
  (chitin, amylose, bioactive pectins from medicinal herb, peptide-mannan etc.)

Colon
- Microflora \((10^{10} - 10^{12} \text{ organisms/g})\)
- Polysaccharides except cellulose are metabolized by microflora into:
  Short chain fatty acids (SCFA) (Acetic acid/Propionic acid/Butyric acid)
Human Peyer's patches (100-250/human)

Peyer's patches: Inductive lymphoid organ in intestine
Jung et al., Int. J. Inflammation (2010, doi:10.4061/2010/823710)
Incorporation of pharmacologically active pectin-type polysaccharides from *Bupleurum falcatum* into Peyer’s patches

Immunohistochemical staining of Peyer's patch by anti-bupleuran 2Ilc/PG-1-IgG after oral administration of BR-2
Regulation of Immune system by Peyer’s patches through common mucosal immune system (CMIS)

- Local mucosa/local lymph nodes
  - Intestine/Colon
  - Upper respiratory tract
  - Genitourinary tract
  - Skin
  - Hemopoietic
  - Other organs (Liver/Pancreas)
  - Brain etc.

Epithelial cell

Plant Polysaccharides (hydrated nanoparticles)

Dendritic cells

Homing receptor-dependent migration

M cells

Interaction

Peyer’s patch

Lymphocytes

Mesenteric lymph node
Assay method for Peyer’s patch-immunomodulating activity

Bone marrow cells

Culture 6 days

Cell proliferation assay

Bone

Peyer’s patch

Intestine

C3H/HeJ mouse

Peyer’s patch cells

Polysaccharides (in vitro)

Conditioned medium

IL-6

GM-CSF etc.

Peyer’s patch-Immunomodulating activity

Hong et al., Phytomedicine, 5: 353-360 (1998)
**Atractylodes lancea DC.**
(medicinal herb)

**Astragalus mongholics** Bunge
(Leaves, health food)

**Glycyrrhiza uralensis** Fisch et DC.
(Licorice, food/medicine)

**Anemarrhena asphodeloides** Bunge
(medicinal herb)

**Phellinus linteus** Aoshima
(Functional food)

**Peyer’s patch-immunomodulating polysaccharides**
Peyer’s patches-immunomodulating activity of screened Plant polysaccharides

Immunomodulating activity against Peyer’s patch cells (Relative fluorescence unit)

- Control
- Astragalus mongholics (Leaves, edible)
- Glycyrrhiza uralensis (Licorice, Edible/medicinal)
- Anemarrhena asphodeloides (medicinal)
- Lentinan
- Atractylodes lancea (medicinal)
- Phellinus linteus (edible)

**p<0.001  ***p<0.000  ***p<0.005

10μg/mL
### Effects of oral administration of polysaccharide fractions from screened plants on cytokine productions of Peyer`s patch cells of aged BALB/c mice

**T lymphocyte-unstimulated Condition (ConA⁻)**

- **Control**
  - *Astragalus mongholicus* (Leaves, edible)
  - *Glycyrrhiza uralensis* (Roots, edible/medicinal)
  - *Anemarrhena asphodeloides* (Rhizomes, medicinal)

**T lymphocyte-stimulated Condition (ConA⁺)**

- **Control**
  - *Astragalus mongholicus* (Leaves, edible)
  - *Glycyrrhiza uralensis* (Roots, edible/medicinal)
  - *Anemarrhena asphodeloides* (Rhizomes, medicinal)

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<th>IL-10</th>
<th>IFN-γ</th>
<th>TGF-β</th>
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<td>P &lt; 0.0001</td>
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<td>Glycyrrhiza uralensis</td>
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<tr>
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<td>P &lt; 0.05</td>
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(100mg/kg/day, 2 weeks)
# Intestinal immune system modulating polysaccharides found in Plants

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<tr>
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<th>Active PS</th>
<th>Relative activity</th>
<th>Class</th>
<th>Active site</th>
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<td>Galactan</td>
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<td>(medicinal)</td>
<td>ALR-a-Bb</td>
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<td>RGII-like PS</td>
<td>Rhamnogalacturonan II</td>
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<td>Arabinogalactan</td>
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<td>Pectin</td>
<td>Rhamnogalacturonan I</td>
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<td>AM-PS-18</td>
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Effect of degradation of β-D-(1→3)-galactan backbone of arabinogalactan from *Atractylodes lancea* on Peyer’s patch-Immunomodulating activity

ex-β-D-(1→3)-galactanase from *Irpex lacteus* (GNase)

G → [3G₆ → 3G₆ → 3G₆ → 3G₆ → 3G₆] →

G₆, G₆, G₆, G₆, G₆, G₆

G = D-Gal

Peyer’s patch- Immunomodulating activity

(Relative fluorescence unit)

Control

No treat

GNase

(100 μg/mL)

P < 0.05
Major structure of galactan moiety in non-reducing terminal side of Peyer’s patch-immunomodulating arabinogalactan (ALR-5IIa-1-1) from *Atractyodes lancea*.

β-D-GlcA-bearing side chains in β-D-(1→3,6)-galactosyl chain in Rhamnogalacturonan I contribute to expression of Peyer’s patch-immunomodulating activity

**Rhamnogalacturonan I (RG-I)**

*Astragalus mongholics*

[Diagram showing the structure of Rhamnogalacturonan I with labeled atoms and enzymes]

**Exo-β-D-(1→3)-Galactanase (GNase)**

**Exo-β-D-glucuronidase (GlcAase)**

**Peyer’s patch-immunomodulating activity**

(Relative fluorescence unit)

- CONTROL
- RG-I
- GNase
- GlcAase

Concentration: 100 mg/mL

P<0.0005

β-D-(1→4)-galactosyl chain in pectic arabinogalactan from Licorice roots contribute to expression of Peyer’s patch-immunomodulating activity.

**Pectic arabinogalactan**

*Glycyrrhiza uralensis* (Licor)
Requirement of oligosaccharide-based cluster (glyco-cluster) formation for expression of modulating activity against Peyer`s patch cells

**β-D-(1→3,6)-galactan chain**

- **Active site**
  - *Atractylodes lancea*
  - *Astragalus mongholics*

**Rhamnogalacturonan I**

- **Astragalus mongholics**
  - **β-D-(1→3,6)-galactosyl chain**

**Glyco-cluster**

- **oligosaccharides**

**Modulation of immunofunction of Peyer`s patch cells**

- **Receptor for oligosaccharides**
  - **signal**

**Active site**

- Rha
- 6-linked
- Gal
- GalA
- 3-linked
- GlcA
Artificial trivalent phenyl-glyco-cluster based on β-D-(1→6)-galacto-mono- to disaccharides bearing 4- O-Me-GlcA terminal

Tetravalent peptidyl-glyco-cluster and dendrimer of \(\beta\)-D-(1\(\rightarrow\)6)-galacto-disaccharides bearing 4-O-Me-GlcA terminal

Glyco-cluster

Glyco-dendrimer

Monomer


Effects of artificial glyco-clusters or dendrimers on function of Peyer’s patch cells from young and aged C3H/HeJ

**Astragalus arabinogalactan**

- 4MeGlcA1→6Gal

**Monomer**

- Trivalent phenyl cluster
  - 4MeGlcA1→6Gal

**Tetravalent peptidyl cluster or dendrimer**


**Peyer’s patch-immunomodulating activity**

- Young C3H/HeJ vs. control
  - ***p<0.0001**
  - **p<0.005**
  - *p<0.05

- Aged C3H/HeJ
  - NS
  - **p<0.005**
  - *p<0.05

Concentration: 100 mg/mL
Carbohydrate chains contribute to Peyer's patch immunomodulating activity

Rhamnogalacturonan II (RG-II)

Arabinogalactan

Rhamnogalacturonan I (RG-I)

Active site

Atractylodes lancea
Astragalus mongholics

Glycyrrhiza uralensis

Astragalus mongholics

β-D-(1→3,6)-galactosyl chain

β-D-(1→4)-galactosyl chain

Glycyrrhiza uralensis

Active site
How do Peyer’s patch-immunomodulating polysaccharides direct to regulate immune system?

Epithelial cell

Peyer’s patch

Immunopotentiation? (Th1/Th2/Th17)

Immunosuppression?

Tolerance?

Mesenteric lymph node

Peyer’s patch

Plant polysaccharides

Dendritic cells

lymphocytes

Interaction?
B10.A mouse and Food antigen-derived IgE

Ovalbumin (OVA)
0.1mg/day/mouse
Oral administration for 2 months

B10.A mouse (♀, 7 weeks old)

Food antigen-derived IgE/type 2 IgG
(IgG4 is important for human, IgG1 for mice)

Pathogenic factors for:
- Food allergy (acute phase)
  5-10% babies in Japan
  9% adults in Japan
- Oral allergic syndrome (OAS)
- Functional dyspepsia
- Irritable bowel syndrome (IBS)
  [Park et al., Neurogastroenterol. Motil., 18, 595-607 (2006)]

Similar antigenic proteins as Pollen antigens are exist in foods
(Cider allergen: Apple, Cherry, Tomato, Carrot etc.)
  [Bohle et al., Allergy, 62, 3-10 (2007)]

OVA-specific IgE/IgG1 induction in blood stream
Akiyama et al., Immunology (2001)

Around 250 mg daily dose for human
Effects of Peyer`s patch-immunomodulating polysaccharides from *Astragalus mongholics* and *Glycyrrhiza uralensis* on IgE induction against ingested food antigen

B10.A mice (Female, 7 weeks old) (n=7〜8)

Ovalbumin (OVA, 0.1 mg/mouse/day, p.o.)

Control (water, p.o.)
Polysaccharide fraction (100mg/kg/day, p.o.)

Serum

- OVA-specific IgE
- Total IgE

**OVA-specific IgE** (Mean±SD, n=7〜8)

**Total IgE** (Mean±SD, n=7〜8)

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<tr>
<th></th>
<th>Normal OVA(-)</th>
<th>Control</th>
<th>Astragalus mongholics (AG, pectic AG)</th>
<th>Glycyrrhiza uralensis (AG, pectic AG)</th>
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<td><strong>OVA(+)</strong></td>
<td>4</td>
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P<0.005

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P<0.005

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p<0.05

p<0.005
Effects of Peyer's patch-immunomodulating polysaccharides from *Phellinus linteus* and *Anemarrhena asphodeloides* on IgE induction against ingested food antigen

B10.A mouse
(Female, 7 weeks old, n=8)

**Ovalbumin (OVA, 0.1 mg/mouse/day, p.o.)**

Control (water, p.o.)
Polysaccharide fraction
(100 mg/kg/day, p.o.)

Anti-OVA-IgE titer ($2^n$)

- Normal
- Control
- *Phellinus linteus*
- *Anemarrhena asphodeloides*
- Konjac glucomannan

**Anti-OVA-specific IgE**

0 2 4 6 8 10 12

- NS; Not significant
- P=0.0626
- P<0.1
- P=0.0114

Branched β-D-(1→3)-heteroglucan

B-D-(1→4)

β-D-(1→3)-heteroglucan

β-D-(1→4)
Effects of Peyer’s patch-immunomodulating polysaccharide from Astragalus mongholics on IgA induction against orally administered antigen

Control (water, p.o.)
Polysaccharide fraction (AG, Pectic AG) from Astragalus mongholics (100 mg/kg/day, p.o.)

OVA-entrapped microcapsule (~5 μm)
(OVA content, 1 mg)
(OVA 1 mg/day, 8 times administration)

Oral administration

BALB/c mouse (♀, 7 weeks old)

OVA-specific IgA induction (intestine)
Tabata et al., Vaccine (1996)
Summary of immunopharmacological activity of different Peyer’s patch-immunomodulating polysaccharides

**Peyer’s patch-immunomodulating polysaccharides**

- *Phellinus linteus* (branched β-D-(1→3)-heteroglucan)
- *Astragalus mongholicus* (AG, pectic AG)
- *Glycyrrhiza uralensis* (Licorice) (Pectic AG)
- *Anemarrhena asphodeloides* (Glucomannan)

**Protective effect of Infection on intestine/Respiratory tract**

- BALB/c mouse (Female, 7 weeks old)
- Healthy mice
- **Up!**

**Control of Diseases related to Food-antigen-Specific IgE**

- B10.A mouse (Female, 7 weeks old)
- **Down-regulated**

**IgA production in intestine/upper respiratory tract**

- **Up!**

**OVA-specific IgE induction**

- **Down!**
Plant pectic polysaccharides have several possibilities for health care.

- Plant polysaccharides
  - Structure-dependent
  - Peyer’s patches
    - regulation
    - Mucosal immune system
      - Infectious Diseases
      - Allergic diseases
      - Brain function
        - ?
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