

## Review

# CAM and NK Cells

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It is believed that tumor development, outgrowth and metastasis are under the surveillance of the immune system. Although both innate and acquired immune systems play roles, innate immunity is the spearhead against tumors. Recent studies have revealed the critical role of natural killer (NK) cells in immune surveillance and that NK cell activity is considerably influenced by various agents, such as environmental factors, stress, foods and drugs. Some of these NK cell stimulants have been used in complementary and alternative medicine (CAM) since ancient times. Therefore, the value of CAM should be re-evaluated from this point of view. In this review, we overview the intimate correlation between NK cell functions and CAM agents, and discuss possible underlying mechanisms mediating this. In particular, neuro-immune crosstalk and receptors for CAM agents are the most important and interesting candidates for such mechanisms.

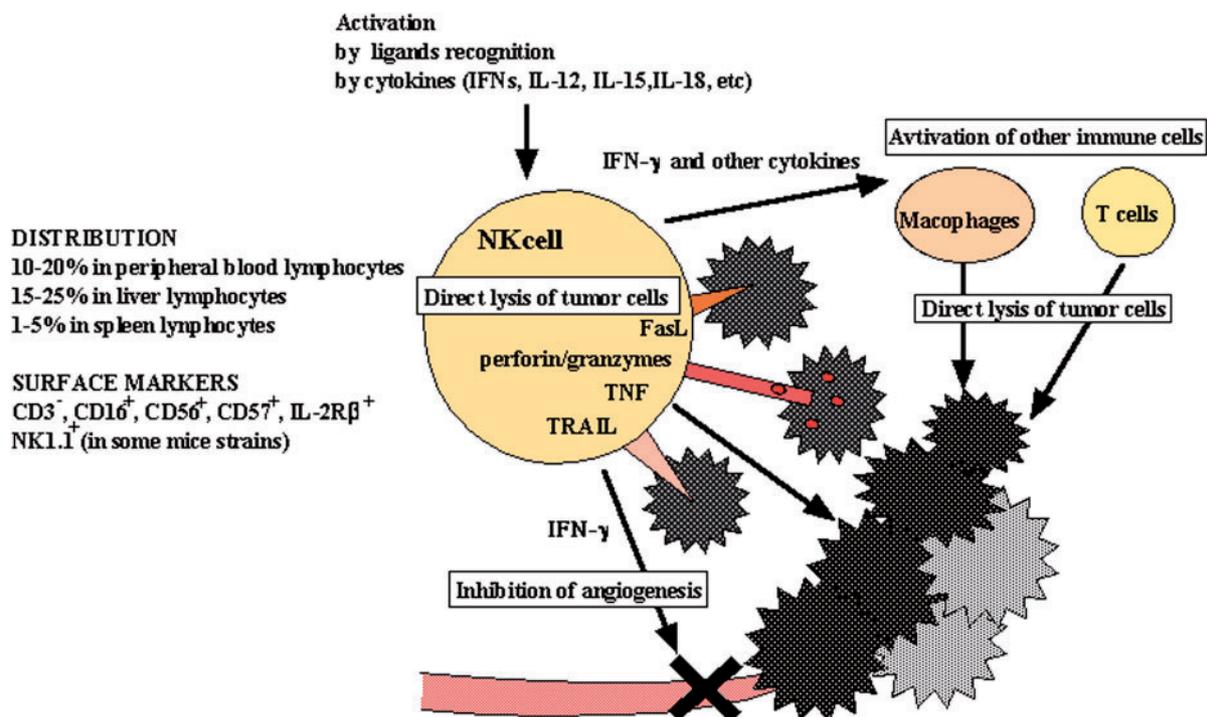
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## Introduction

It has been well known since ancient times that complementary and alternative medicine (CAM), including exercise, provides a lot of benefit to health. Many CAM modalities are believed to prevent or even cure diseases, especially morbid ones such as cancer. However, until recently conventional medicine has largely rejected the use of CAM agents because little biological evidence has been provided for the functional mechanisms of many of them. For the past few decades, the value of CAM has been rediscovered by many modern scientific researchers. Modulation of immune functions by CAM agents is the mechanism most widely analyzed and has been suggested to provide some scientific evidence for the biological effects of various CAM agents. Specifically, evidence for up-regulation of natural killer (NK) cell numbers and/or NK cell cytotoxicity by these agents has been accumulating. NK cells have been well established as innate cytotoxic effector cells for self defense in both vertebrates and invertebrates, in both of which they may use similar mechanisms in the lysis of target cells (1). In this review we overview NK cell functions and their functional molecules, particularly in relation to cancer, and discuss the possible mechanisms of NK cell activation by various CAM agents.

## Why are NK Cells Important?

Ever since Lewis Thomas and Macfarlane Burnet proposed the immune surveillance hypothesis against tumor development, the concept has been a hot focus of debate for more than 40 years (2,3). The original immune surveillance hypothesis was challenged because nude mice lacking T cells did not show a higher incidence of cancer than did syngenic immunocompetent mice (4). However, this contradictory conclusion is now taken as fine evidence that innate immunity is in the critical arms of immune surveillance against tumor development. Moreover, in the innate immune system NK cells, which do not express T-cell receptors that recognize specific peptides presented on the major histocompatibility complex (MHC), rather than T cells, seem well suited for this role. NK cells thus mediating natural cytotoxicity are composed predominantly of large granular lymphocytes (LGL) and some of small agranular lymphocytes, both of which express CD16 and CD56, but no T-cell receptor, on the cell surface (5,6). NK cells can induce cytolysis in the absence of MHC class I antigen expression on their target cells (5). This phenomenon is commonly understood according to the 'missing self' hypothesis (7). Culture of NK cells with some cytokines augments their cytotoxic activity so that they become able to induce cytolysis for a wide spectrum of cells, including tumor cells expressing autologous MHC class I. They are now called lymphokine-activated killer (LAK) cells, although LAK cells are also induced by T lymphocytes (8–10).



**Figure 1.** NK cells in tumor surveillance. NK cells are activated by some cytokines and direct recognition of ligands of their NK cell activating receptors, which result in direct tumor cell lysis through their cytotoxic molecules (perforin/granzyme, Fas ligand, TRAIL) and activation of other immune cells (macrophages and T cells) through cytokine production. IFN- $\gamma$  produced by NK cells also plays a critical role in the inhibition of angiogenesis by tumor development.

## NK Cells and Cancer

### Tumor Immunosurveillance by NK Cells in Animal Models

Animals with low levels of NK cell activity (beige, cyclophosphamide or anti-asialo GM1 antibody-treated mice) have been shown to develop an increased number of spontaneous and experimental tumors and their metastases (11–13). Conversely, animals with augmented NK cell activity (treated with *Corynebacterium parvum* or IFN-inducer) display increased resistance to the development of metastasis (14). Adoptive transfer of splenocytes with high NK cell activity partially restored the anti-metastatic ability of anti-asialo GM1 and cyclophosphamide-treated animals (12,15). Direct evidence for the role of LGL in the prevention of blood-borne metastases was demonstrated by the inhibition of experimental metastases in NK cell-deficient mice following adoptive transfer of LGL (16,17).

### Tumor Immunosurveillance by NK Cells in Humans

Despite relevant evidence from animal studies, the role of NK cells in immunologic surveillance against cancer in humans is poorly defined. Circumstantial evidence for the crucial role of natural cytotoxicity in human cancer was obtained by studying patients with NK deficient states. Patients who have Chédiak–Higashi syndrome (18), X-linked lymphoproliferative syndrome (19) or who have had renal transplants (20) all have reduced NK cell activity and an increased incidence of

cancer. Various studies have shown peripheral blood NK cell activity to be significantly reduced in patients with cancer compared with non-cancer-bearing control subjects (21–23). Other researchers, however, have documented no reduction in NK cell activity in patients with some cancers, irrespective of the stage of the disease (24,25). Thus, the precise role of NK cell cytotoxicity in human cancer remains inconclusive. Overall, however, there appears to be a trend for NK cell activity to be reduced in certain tumors, possibly related to tumor volume or dissemination. Some studies reported an increased risk of developing cancer in individuals with low NK cell activity, and normal NK cell activity only in the early stage of cancer (21,26–28). Several groups have reported increased blood-borne metastasis, significantly increased risk of death from uncontrolled regional and distant metastasis, the development of regional and distant metastasis and risk of local recurrence in patients with low NK cell activity (29–33). Moreover, one epidemiological study recently indicated that low NK cell activity is associated with increased cancer risk, although it is premature to conclude from this result that NK cell activation induces tumor prevention (34). However, even this report can not be taken as clear evidence for NK cell-dependent tumor surveillance in humans, since expression of MHC class I, the most critical molecules for susceptibility to NK cell activity, on developing tumors was not analyzed.

We suggest that natural cytotoxicity mediated by NK cells may have a role in the prevention of the development of cancer and in the establishment of metastasis in humans as well

**Table 1.** Summary of known NK cell receptors

Species	Receptor	Ligands
Mice		
Inhibitory	Ly49	H-2K, H-2D
	CD94/NKG2A	Qa-1 <sup>b</sup>
Activating	CD16	IgG
	NKp46	Influenza haemagglutinin, others?
	NKR-P1C	?
	CD94/NKG2C	Qa-1 <sup>b</sup>
	Ly49D	H-2D <sup>d</sup>
	Ly49H	MCMV-induced?
	NKG2D	RAE1, H60, Mult1
	CD244	CD48
Human		
Inhibitory	KIR2DL	
	KIR3DL	HLA-C
	CD94/NKG2A(CD159a)	HLA-Bw4, HLA-A
Activation	CD85j, CD85d	HLA-E
	CD16	HLA Class I
	NKp30	IgG
	NKp46	?
	KIR2DS	Influenza hemagglutinin, others?
	KIR2DL	HLA-C, others
	CD94/NKG2C	HL-G
	NKp44	HLA-E
	NKG2D	Influenza hemagglutinin, others?
	CD244	MICA/MICB, ULBP
		CD48

HLA, human leucocyte antigen; KIR, killer cell immunoglobulin-like receptors; MCMV, mouse cytomegalovirus; MIC, MHC class I-chain related molecules; RAE-1, retinoic acid early inducible 1; ULBP, UL-16-binding protein.

as mice, although more evidence at the molecular level needs to be accumulated to define the role of NK cells in tumor surveillance in humans. Moreover, further epidemiological studies are needed to more precisely define whether NK cell activity can predict further cancer development in patients at high risk, or if NK cell activity has prognostic value in cancer patients. An intriguing possibility is prevention and/or cure of cancer by augmenting NK cells by CAM agents.

### Cytotoxic Molecules of NK Cells and Tumors

Several studies have demonstrated an increased tumor incidence in mice deficient in cytotoxic molecules. A higher incidence of tumors has been noted in mice deficient in perforin (35,36), which is the critical cytotoxic molecule of NK and cytotoxic T cells (37), and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) (38,39). A higher spontaneous and methylcholanthrene (MCA)-induced tumor formation were also reported in STAT-1 [a signal molecule require for responsiveness to interferons (IFNs)], IFN- $\gamma$ -, and IFN- $\gamma$  receptor-deficient mice (40–43). These show that

impairment of the receptor for cytokines activating NK cells results in defective immune surveillance against tumors. Although these studies provide clear evidence for immune surveillance, there have been few reports of exactly how the tumor cells are recognized as abnormal and then eliminated. Recent studies on NK cell receptors have indicated the possible receptors responsible for tumor recognition and thus provide new support for the hypothesis of NK cell-mediated tumor surveillance (Fig. 1).

### NK Cell Receptors for Tumor Recognition

The first NK cell receptors clearly defined were shown to bind both classical and non-classical MHC class I molecules and block the killing of target cells by NK cells (44). This inhibition was associated with immunoreceptor tyrosine-based inhibitory motif (ITIM) domains in the cytoplasmic tails of these NK receptors. ITIMs provide docking sites for phosphatases that oppose the activity of tyrosine kinases, which are essential enzymes for NK cell activation. However, certain members of the NK receptor family, KIR2DS, Ly49D/H and CD94/NKG2C, do not possess ITIMs. Instead, they are associated with the adapter molecule DAP12, which has an immunoreceptor tyrosine-based activating motif (ITAM) domain capable of activating NK cells (44). Thus, the view is now emerging that the activity of NK cells is regulated by the balance between activating and inhibitory signals. However, other than the down regulation of MHC class I molecules, it has been unclear which other molecules are likely to tip the balance in favor of NK cell activation for tumor surveillance (Table 1).

### The Role of NKG2D in Tumor Surveillance

NKG2D, encoded within the NK receptor gene complex, might be the activating receptor of NK cells critical for immune surveillance against tumors, since some NKG2D ligands (MICA and MICB in human, Rae1 and H60 in mice) can be induced by environmental stress and are expressed on many tumor cells (45–47). Moreover, not only NK cells but T cells expressing the  $\gamma\delta$  or  $\alpha\beta$  T cell receptor (TCR) express NKG2D, thus NKG2D turns out to be the missing link and unites innate and adoptive immunity in immune surveillance and anti-tumor immunity. Presumably, tumor cells, once they express NKG2D ligands, become able to induce immunological tolerance against attack from NK cells, as they are highly enhanced inhibitory ligands to be expressed.

### CD94/NKG2A and CD94/NKG2C for Surveillance of 'Modified Self'

CD94/NKG2 is the other crucial receptor for NK cell-mediated immune surveillance (44,48,49). HLA-E (Qa-1<sup>b</sup> in mice) is the relatively non-polymorphic ligand of inhibitory CD94/NKG2A and activating CD94/NKG2C. It has a preference for nonameric peptide, derived from the signal sequence of other class I molecules. Thus, CD94/NKG2A plays a critical

**Table 2.** Summary of NK cell activation-inducing CAM

CAM	Immunological effects
Green tea	Humoral and cell-mediated immunity including NK cell activation
Ginseng	Increase in macrophage, NK, T and B cells number
Vitamin supplementation	Increase in antibody titer
Honey	Increase in antibody titer
<i>Lactobacillus casei</i> strain Shirota	Increased NK cell activity
Extract	
Aged garlic	Prevent a reduction of NK cell activity by psychological stress; prevent the decrease of spleen weight by psychological stress
<i>Viscum album</i> (mistletoe)	Increased NK cell number and NK cell activity
<i>Cichorium intybus</i>	Increase in circulating leukocytes; increase in phagocytic activity, NK cell activity, proliferation and IFN- $\alpha$ production
<i>Echinaces purpureae</i> root	Increased NK cell activity
<i>Derris scandens</i>	Increased NK cell activity in HIV-infected individuals
<i>Nigella sativum</i> seeds	Increased NK cell activity
<i>Allium sativum</i> bulb	Increased NK cell activity
<i>Onopordum acanthium</i> stem and leaves	Increased NK cell activity
<i>Allium cepa</i> bulbs	Increased NK cell activity
Chinese herb (e.g. Shikaron)	Increased NK cell activity
<i>Phyllanthus emblica</i>	Increased NK cell activity and antibody dependent cellular cytotoxicity (ADCC)
Mushrooms (e.g. <i>Lentinus edodes</i> and <i>Agaricus blazei</i> )	Increased NK cell activity
Physiological	
Acupuncture	Increase in T cell and NK cell numbers; increase in monocyte phagocytosis
Electroacupuncture	Increased NK cell activity and IFN- $\alpha$ and IL-2 production
Skin rubdown	Increased NK cell activity
Exercise (dependent on time and frequency)	Increased NK cell activity and NK cell numbers
Psychological	
Relaxation	Increased NK cell activity and T cell response; increase in the number of peripheral blood lymphocytes
Message therapy	Increase in NK cell number in HIV-infected individuals
Music therapy	Increase in NK cell number and NK cell activity
Mirthful laughter	Prevents a reduction of NK cell activity by psychological stress; increased NK cell activity; increase in NK cells, T cells, immunoglobulins and IFN- $\alpha$ production

role in blocking NK cell cytotoxicity by classical and non-classical MHC class I molecules. It might also be the receptor most likely to be responsible for the phenomenon explained by the ‘missing self’ hypothesis: namely, loss of MHC class I expression leads to sensitivity to lysis by NK cells (7). It was recently reported that a peptide from the signal sequence of stress protein hsp60 is loaded onto HLA-E, competing effectively with an MHC class I-derived peptide, and up-regulates surface expression of HLA-E (50). HLA-E loaded with the hsp-derived peptide does not bind to CD94/NKG2A. Therefore, stress-induced modification of HLA-E would activate CD94/NKG2A-expressing NK cells due to loss of inhibitory ligands, which would result in the augmentation of sensitivity to NK cells on the part of cells expressing hsp60. ‘Modified self’ (ligand modification of inhibitory receptor: CD94/NKG2A,HLA-E) is another mechanism by which susceptibility of tumor cells to NK cells is augmented, in addition to the ‘missing self (reduced class I expression)’ and ‘induced self’ (expression of NKG2D ligands) mechanisms (51).

## NK Cells and CAM

### Activation of NK Cells by CAM Agents

For the past few decades, scientific investigations on CAM have remarkably advanced and partly supported their medical efficacy through preclinical and clinical experiments. Such CAM modalities include: Oriental medicine, especially traditional Chinese medicine (including Kampo and acupuncture), extract products from natural plants, animal molecules and live lactic acid bacteria. In particular, many investigators have suggested that NK cell activation is one of the critical mechanisms for the biological effects induced by various CAM agents. For example, intake of green tea and some kind of live lactic acid bacteria enhanced NK cell activity (52–54). Administration of extracts from ginseng (52), aged garlic (55), *Viscum album* (mistletoe) (56,57), *Cichorium intybus* (58), *Echinacea purpurea* root (59), *Derris scandens* hydroalcoholic (60), some wild plants (61),

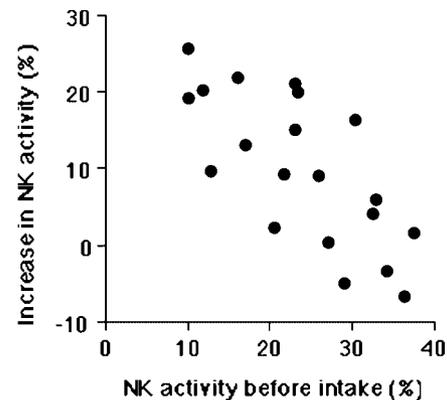
Chinese herbs (62) and some kinds of mushrooms (63) significantly augmented NK cytotoxicity or restored NK cell activity in some immune-suppressive conditions. Some of these agents showed inhibition of experimental metastasis of cancer. Oral administration of *Phyllanthus emblica*, which is known as an excellent source of vitamin C, enhanced NK cell activity and antibody-dependent cellular cytotoxicity (ADCC) (64), thus, supplemental nutriment might enhance NK cell activity. Moreover, acupuncture (65,66), skin rub-down (67), relaxation (68,69), message therapy (70), music therapy (71), mirthful laughter (72,73) and hypnotherapy (74) enhanced NK cell activity and/or NK cell numbers. There are a considerable numbers of studies reporting that acute and chronic exercise and long-term repeated exercise (training effects) elevated NK cell activity in peripheral blood (75–79) (Table 2).

Thus, numerous studies have reported the augmentation of NK cell number and/or NK cell cytotoxicity by CAM agents as described above. In order to examine this important issue further, however, criteria for the quality of evidence need to be established. It would not mean much, for example, if a report only showed that a certain CAM agent increased the number of NK cells in mice or humans.

A considerable number of reports have presented clinical observations. However, large-scale epidemiological studies to establish intimate correlations of any single CAM agent with NK cells in any specific disease conditions (e.g. cancer) are still lacking. Future epidemiological studies should be based on sound biological experiments both *in vitro* and *in vivo*. In selecting parameters for prospective epidemiological studies, we would like to draw attention to the next point below, and also the problem of individual differences between possible 'CAM-agent receptors' as shown in the following sections. Without such biological considerations, epidemiological analysis will be pointless.

It is now well known that NK cell activity is regulated by expression levels of cytotoxic molecules, activating receptors and/or inhibitory receptors. Thus, decrease or increase in NK cell number in peripheral blood mononuclear cell (PBMC) does not necessarily result in a decrease or increase in NK cell activity, respectively. Therefore, in designing any further experiments in this field, NK cell numbers and NK cell cytotoxicity should be analyzed simultaneously, and responsible molecules or mechanisms for CAM effects should be clearly defined.

Cytotoxic and activation mechanisms of NK cells have been demonstrated in experimental models using various gene-modified mice (transgenic and gene-knock-out mice). Therefore, we recommend researchers to design experiments using such laboratory mice. It should become the standard preclinical system in which mechanisms of the effects of any CAM agents in the activation of NK cells are to be clearly demonstrated *in vivo*.



**Figure 2.** Intimate correlation between the magnitude of the increase in NK cell activity and the level of NK cell activity. The increase in NK cell activity after the intake of LcS drink is prominent in individuals with low NK cell activity. The correlation was determined by Pearson's methods; statistical significance was  $P < 0.05$ .

#### **Example: NK Cell Activation by *Lactobacillus casei* Strain Shirota and Extract of Mushrooms**

We have analyzed the effect on immunological function of a fermented milk drink containing *Lactobacillus casei* strain Shirota (LcS) (53). The frequency and the number of NK cells, CD4<sup>+</sup> T cells or CD8<sup>+</sup> T cells were not significantly changed, and T cell responsiveness to mitogens (Con A and PHA) and allantigen stimulation were unchanged. Moreover, significant increase of IFN- $\alpha$  and IFN- $\gamma$  could not be detected in serum at all. However, NK cell cytotoxicity was significantly increased, and the enhancement of NK cell activity was particularly prominent in the low NK activity individuals (Fig. 2). We have also analyzed NK cell activity after the intake of the extract of mushrooms, *Agaricus blazei* and *Lentinus edodes*, which are commonly used CAM agents in Japan. Although intake of these extracts did not induce significant change in lymphocyte populations and T cell responses, NK cell activity was augmented. Notably, susceptibility to extract of *Agaricus blazei* was varied among individuals; however, it was closely correlated to the responsiveness to the extract of *Lentinus edodes* but not LcS. This suggests that these individuals are selectively susceptible to some general components of the mushroom extracts (Table 3). Conversely, NK cell activity was highly augmented by LcS but not the mushroom extracts in some volunteers, and only slight augmentation of NK cell activity was observed by either LcS or mushrooms extract in some others, even if their NK cell activity was relatively low.

It has often been suggested that effects of some CAM modalities are varied among individuals. Our examples presented above suggest that the individual differences in the responsiveness to CAM agents may be due to the individual difference of expression of various CAM receptors. If it really is the case, it supports the concept that proper selection of CAM agents for each individual is needed in order for their effects to be manifested.

**Table 3.** Intimate correlation of susceptibility to extracts of mushrooms

	<i>Agaricus blazei</i> *	<i>Lentinus edodes</i> *	<i>Lactobacillus casei</i> strain Shirota	Population
Group A	++	++	various	25%
Group B	+	+	various	50%
Group C	-	-	various	25%
Group D	++ or +	-		0%
Group E	-	++ or +		0%

\*++ , 20% or more increase of NK cell cytotoxicity at E/T = 20; +, around 10% increase in NK cell cytotoxicity at E/T = 20.

### How do CAM Agents Activate NK Cells?

The precise mechanisms of NK cell activation by CAM agents have not been clearly defined. The two main possibilities are: (i) augmentation of cytotoxic molecules in NK cells and/or (ii) up-regulation of activating NK cell receptors and/or down-regulation of inhibitory NK cell receptors. On the other hand, improvement of general 'health' conditions by some CAM agents, e.g. exercise, may result in NK cell activation, although precise pathways are still unclear. We shall discuss in this section two interesting mechanisms, one indirect and the other direct, which seem to be reasonable explanations for the NK cell activation induced by CAM agents.

#### *Neuro-immune Crosstalk*

A growing body of evidence shows a crucial role of the autonomic nervous system in modulating the immune response in stress (75). For example, the autonomic nervous system might play an important role in the modulation of the immune response after both acute and chronic exercise.

Intimate crosstalk between the central nerve systems and the immune systems has been demonstrated by experiments using electrical stimulation of the ventromedial horn of the hypothalamus, which is the site in the central nervous system responsible for delivering sympathetic stimulus to spleen (80,81). There is also evidence to show a crucial role of the autonomic nervous system in modulation of the immune response, especially under the influence of stressors and exercise (82,83), although little is known about the role of parasympathetic nerves compared to the large amount of information available on sympathetic nerves in immune regulation.

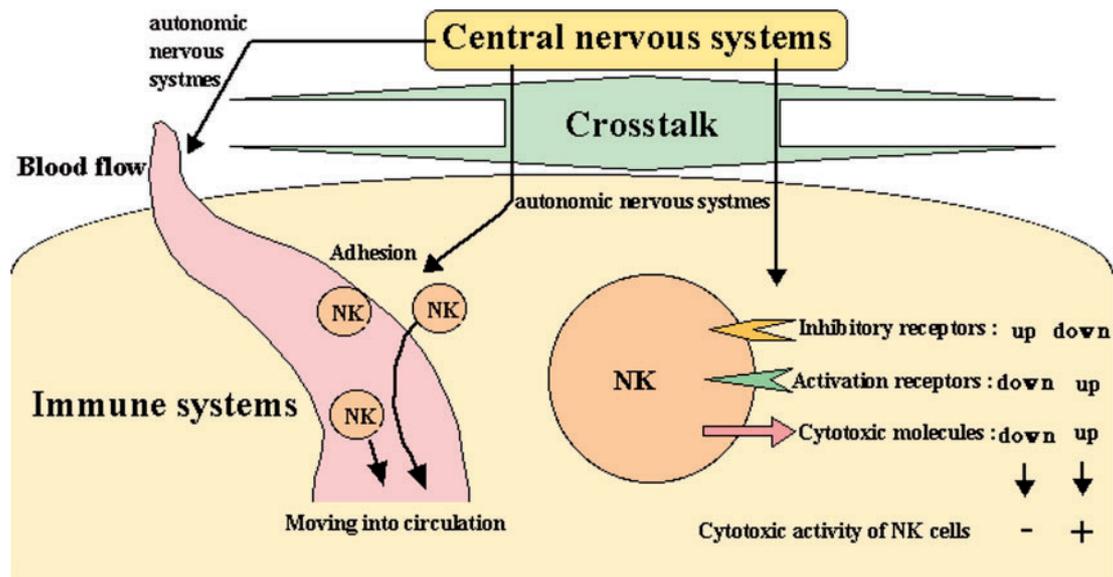
Modulation of the immune response can be mediated either through innervation by sympathetic nerves or through the receptors for their chemical transmitters on immune cells in these organs, as described below.

*Regulation of Blood Flow by the Sympathetic Nervous System*—An important role of autonomic nerves in both primary and secondary lymphoid organs was reported for the regulation of blood flow (81,84,85), which plays a major role in immune regulation by facilitating the right cells to lodge or move to where they can efficiently accomplish their task (86,87).

*Direct Effects of the Sympathetic Nervous System on Immune Cells*—A growing number of studies suggests that sympathetic nerves can influence immune cells more directly than just by regulating the blood flow. Catecholamines released from sympathetic terminals are major mediators responsible for immune regulation when they bind to their specific receptors (88). Their suppressive nature on NK cell cytotoxicity has been reported (89,90). In striking contrast, acute deprivation of sympathetic innervation causes enhancement, while its chronic deprivation seems to cause suppression, of NK cell activity, and diminished cell-mediated immunity (91,92).

*Impact of Sympathetic Modulation on the Distribution of Leukocytes*—An active involvement of autonomic regulation in leukocyte distribution is suggested by the following fact. The increase in circulating NK cells and decreased NK cell attachment to endothelial cells induced by catecholamines but independent of adhesion-molecules, was inhibited by  $\beta$ 2-adrenergic antagonist (93–95). NK cells have higher expression of  $\beta$ -adrenergic receptors and lower expression of cholinergic receptors, and are thereby influenced by sympathetic and parasympathetic activities (96). Mental stress accompanied by sympathetic activation increases the number of NK cells in circulation, which is blocked by  $\beta$ -adrenergic antagonist (93,97,98). In the same study it was demonstrated that spleen does not serve as a pool for NK cells (93). On the other hand, an increased accumulation of catecholamine-stimulated lymphocytes to secondary lymphoid organs might account for the transient decrease in lymphocytes from circulation after catecholamine injection (99). Thus, the origin of NK cells released into circulation after  $\beta$ -adrenergic stimulation is an open question yet to be answered.

Taken together, the sympathetic nervous system does not simply suppress the immune system but might help in organizing the immune response sequentially and spatially by modulating the distribution of immune cells. Modification of blood flow and cell adhesion must be the crucial event for increasing NK cells in peripheral blood by CAM agents, since increase of NK cell numbers does not always link to augmentation of NK cell cytotoxicity (Fig. 3).



**Figure 3.** Summary of immune system control by autonomic nervous system. The central nervous and immune systems intimately crosstalk in the live body. In particular, sympathetic nerves mediate inhibitory effects on NK cells. NK cell distribution and NK cell activity are controlled by autonomic nervous systems directly and indirectly.

#### Receptors for CAM Agents on NK Cells?

Control of the immune functions mediated by the nervous system would be the major mechanism underlying NK cell activation by physical CAM modalities (e.g. exercise, skin rubdown and acupuncture) and psychological CAM ones (e.g. relaxation, message therapy, music therapy and mirthful laughter). On the other hand, numerous CAM agents have also been reported to activate NK cell cytotoxicity, improve general health and reduce the risk of cancer development. This type of phenomena, if real, should be understood as reasonable if NK cells and/or some innate immune cells expressed receptors for some components of various types of CAM agents (e.g. liquid and food). Toll-like receptors and receptors to lectins and  $\beta$ -glucan polysaccharides are the most likely candidates responsible for NK cell activation induced by intake-type CAM agents (100). These CAM-agent receptors may well be preferentially expressed on macrophages or dendritic cells. In such cases, these CAM-agent-responding cells produce cytokines (e.g. type-I IFN, IL-12 and IL-18), which induce NK cell activation.

**Toll-like Receptors Involved?**—Toll-like receptors (TLRs) function as the pattern-recognition receptors in mammals and play an essential role in their recognition of microbial and fungal components (101–104). Ten members of the TLR family have been identified in humans. TLRs activate NK- $\kappa$ B and other signaling pathways, which results in the secretion of various inflammatory cytokines (101–105). It has been reported that TLR-2/6 recognize some components of zymosan, but not  $\beta$ -glucan, which result in production of cytokines and chemokines (106,107). Thus, TLRs might play important roles in the biological effects of some intake-type

CAM agents. This is a very interesting subject for further studies.

**Receptors for Lectin Involved?**—It has been established that various members of the lectin family modify immunological functions (108,109). Wild plants and fungi have traditionally been the single largest source of lead lectin compounds for the development of therapeutics agents by the pharmaceutical industry. Currently, mushroom and plant polysaccharides are the focus of attention in relation to CAM, stimulating scientific analysis and drug development to prevent and treat cancer. Thus, some unknown C-type lectin-like receptors for certain components of CAM agents and/or lectin, and lectin interaction, might play roles in CAM effects.

**Receptors for  $\beta$ -Glucan Involved?**—The *in vivo* administration of  $\beta$ -glucan has been shown to potentiate host responses against a variety of conditions, including tumor development and infection (100,110,111). This has led to a number of clinical trials using  $\beta$ -glucans for tumor immunotherapy with some promising results. Thus,  $\beta$ -glucan receptors are one likely class of receptors responsible for NK cell activation by CAM agents.

The  $\beta$ -glucans are a heterogeneous group of glucose polymers, consisting of a backbone of  $\beta(1\rightarrow3)$ -linked  $\beta$ -D-glucopyranosyl units with  $\beta(1\rightarrow6)$ -linked side chains of varying distribution and length. These polysaccharides are major cell wall structural components in fungi, mushrooms, plants and some bacteria. As they are not found in animals, these carbohydrates are considered to be classic pattern-recognition molecules (101) and are recognized by the innate immune system. Vertebrate recognition of  $\beta$ -glucans appears to occur exclusively via several cell surface receptors, and although

**Table 4.** Summary of immunostimulating polysaccharides of higher basidiomycetes

Polysaccharide	Species
Glucans	
$\alpha$ -(1→6)-; $\alpha$ -(1→4)-glucan	<i>Agaricus blazei</i>
$\alpha$ -(1→4)-; $\beta$ -(1→6)-glucan	<i>Agaricus blazei</i>
$\beta$ -(1→6)-; $\alpha$ -(1→3)-glucan	<i>Agaricus blazei</i>
$\beta$ -(1→6)-; $\beta$ -(1→3)-glucan	<i>Agaricus blazei</i>
Galactomannoglucan	<i>Ganoderma tsugae</i>
Galactoxyloglucan	<i>Hericium erninaceus</i>
Glucan phosphate	<i>Saccharomyces cervisiae</i>
Grifolan	<i>Grifola frondosa</i> (Maitake mushroom)
Lentinan	<i>Lentinus edodes</i> (Shiitake mushroom)
Mannogalactoglucan	<i>Pleurotus pulmonarius</i> <i>Agaricus blazei</i>
PGG-Glucan	<i>Saccharomyces cervisiae</i>
Riboglucan	<i>Agaricus blazei</i>
Schizophyllan	<i>Schizophyllum commune</i>
Scleroglucan	<i>Sclerotium glukanicum</i>
SSG-Glucan	<i>Sclerotinia sclerotiorum</i>
Xyloglucan	<i>Grifola frondosa</i> <i>Pleurotus pulmonarius</i>
Zymogen	<i>Saccharomyces cervisiae</i>
Glycan	
$\beta$ -(1→2)-; $\beta$ -(1→3)-glucomannan	<i>Agaricus blazei</i>
Galactoglucomannan	<i>Lentinus edodes</i> (Shiitake mushroom)
Glucogalactan	<i>Ganoderma tsugae</i>
Glucomannan	<i>Agaricus blazei</i>
Glucosylyan	<i>Hericium erninaceus</i> <i>Pleurotus pulmonarius</i>
Mannogalactan	<i>Pleurotus pulmonarius</i>
Mannogalactofucan	<i>Grifola frondosa</i>
Mannoglucoxylyan	<i>Hericium erninaceus</i>
Xylan	<i>Hericium erninaceus</i>

complement opsonization does contribute to the recognition of particulate glucans, no plasma molecules recognizing this carbohydrate structure have been identified.  $\beta$ -Glucan recognition systems in invertebrates are completely different from vertebrates (112); however, the recognition of  $\beta$ -glucan by both systems results in the triggering of innate immunity (Table 4).

$\beta$ -Glucan receptor activity has subsequently been reported on a variety of leukocytes, including macrophages, neutrophils, eosinophils and NK cells, as well as on non-immune cells including endothelial cells, alveolar epithelial cells and fibroblasts. Non-opsonic recognition of  $\beta$ -glucan by these cells has been ascribed to multiple receptors (113), and indeed a number of  $\beta$ -glucan receptors have been identified, including CR3 (110), lactosylceramide (CDw17) (114), scavenger receptors (115) and dectin-1 (116–118). Of these receptors, however, dectin-1 has been clearly shown to play

a role in mediating the biological response to  $\beta$ -glucan (119). Although the mechanisms are not yet fully understood, the generation of the response starts at the cell surface and requires a functional ITAM domain in cytoplasmic tails of dectin-1. The ITAM motif becomes phosphorylated after  $\beta$ -glucan binding, suggesting that signaling might occur in a similar fashion to that of other ITAM-containing receptors, such as NK-activating receptors and Fc receptors.

Not only  $\beta$ -glucan polysaccharides, but also oligosaccharides have been reported as the effector components of some CAM agents, which down-regulate production of immunosuppressive cytokines (100). It has been suggested that glycochain, glycopeptide and glycolipid play a critical role in numerous biological events, including immunological responses (120–125). However, glycobiology still cannot clearly reveal the molecular mechanisms for their biological functions. Further analysis in glycobiology will identify the crucial receptors for CAM on immune cells and elucidate the essential molecules and mechanisms. As this field is rapidly progressing, our perspective may be drastically changed in several years. It will be interesting for us to present a revised and expanded version of this review in a couple of years.

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