Enhancement and modulation of the immune system by melanin

A number of previous studies have shown that both plant□ and synthetic melanin can modulate cytokine production and enhance several immune parameters 24-27.

Recently, it has been demonstrated that animal and fungus melanin (derived from rat and Aspergillus fumigatus, respectively) also modulates cytokine production 28, 29. Sava et al. (2001) 21 extracted melanin□like pigments (MLPs) from black tea and showed that oral administration of MLPs to mice significantly stimulated splenic lymphoid tissue. Later, Hung et al. demonstrated that melanin extracted from different tea species induced cytokine production, with green tea melanin being at least 100 times more active than black tea melanin 30. They have also reported that antibody□secreting cells produced significantly more antibodies in animals treated with tea melanin (32–34%) than did antigen controls. Similarly, Al□Mufarrej et al. (2006) 31 showed that, in albino rats, black seed melanin induced a high and long□lasting antibody response to sheep red blood cells by stimulating the immune system. The immunostimulatory effects of melanin preparations from 30 traditional medical herbs were studied and patented by Pasco et al. (2005) 32. The patent authors reported that the melanins with the highest levels of activity were found in Allium sativum, Tabebuia spp., Serenoa repens and Echinacea spp.

Pugh et al. (2005) 24 demonstrated that ingestion of these melanins by mice caused dendritic cells in Peyer's patches to secrete high levels of IgA and interleukin□1 (IL□1). In these studies, spleen cells from mice that were fed melanins exhibited increased production of interferon gamma (IFG□□) as a result of a shift in the balance of T helper 1 and T helper 2 cells (Th1 and Th2, respectively) in favour of Th1. Avramidis et al. (1998) 33 found that grape melanin modulates the production of IL□1, IL□6 (interleukin□6) and tumour necrosis factor□α (TNF□α) and significantly inhibited adjuvant□induced disease in rats. They suggested a possible role for melanin in inhibiting lymphocyte Th1 (T4 or T8), which led to the suppression of adjuvant□induced disease development. Recently, the important role of Toll□like receptors (TLRs) and their corresponding ligands in modulating cytokine production has been reported 34. TLRs are transmembrane or cytoplasmic receptors that recognize conserved molecular patterns of bacteria, fungi and viruses. They are members of the IL□1 receptor superfamily and share significant homology in their cytoplasmic regions 35, 36.

The most well□defined exogenous ligands for TLRs are lipopolysaccharide (LPS) for TLR4 and TLR2 and peptidoglycans and lipoproteins for TLR2 37, 38. In addition to exogenous ligands, TLR4 and TLR2 can be stimulated by various endogenous ligands such as heat□shock proteins (HSPs) and fibronectin 39-41.

Plant melanins represent a new class of polymers that are recognized by the TLR family. Melanin isolated from Echinacea and Nigella sativa seeds were shown to activate monocytes by binding TLR□2 and TLR□4, respectively 42. Ligand binding to TLRs induces dimerization and recruitment of various adaptor molecules, which induce the production of various cytokines and activate downstream signalling pathways such as the myeloid differentiation factor 88 (MyD88) pathways. These cascades primarily include the nuclear factor□kB (NF□kB), MAP□kinase and interferon regulatory factor□3 and interferon regulatory factor□7 (IRF□3 and IRF□7) signalling pathways. In addition, TLR4 activation can induce a MyD88□independent pathway, TRIF, and activate the IRF□3 pathway 43 (fig. 1).

TLR signalling pathways. Transmembrane TLRs (represented here by TLR4) recognize external ligands (exogenous and endogenous), whereas cytoplasmic TLRs (TLR3) recognize intracellular signals. When activated, the majority of TLRs induce activation of NFκ□B (early□phase activation) and cytokine production in a MyD88□dependent manner. However, TLR4, like TLR3, can also signal in a MyD88□independent manner and induce expression of type I interferon (IFN) and IFN□inducible proteins in addition to late□phase NFκ□B activation 43.

Melanin extracted from Nigella sativa L. was shown to directly activate TLR□mediated signalling pathway in monocytes, peripheral blood mononuclear cells and in the THP□1 human monocytic cell line 44. Recently, Muller et al. (2013) 28 demonstrated that melanin extracted from murine melanoma B16F1 cells increased secretion of chemokines MIP□1β (CCL4) in dendritic cells derived from primary monocytes and human monocytic cell line (MoDCs and THP□1, respectively). Kunwar A. et al. (2012) 11 reported that melanin isolated from the fungus Gliocephalotrichum simplex reduced oxidative stress in hepatic tissue and abrogated immune imbalance by reducing the production of cytokines (IL6 and TNF□α) in BALB/C mice.

Most botanicals with immune□enhancing properties contain high molecular weight LPSs or lipoproteins. Consequently, much of their in vitro macrophage□activating properties may be a result of contamination with these agents 22. However, subjecting botanical extracts to high alkalinity or high acidity destroys the LPSs 45-48 and causes subsequent loss of their biological activities, including cytokine modulation 49, 50. A number of studies have shown that melanin treated extensively with acids (pH 2) and alkali solutions (pH 14) still maintains their immunogenic properties 51. Conversely, it is well known that melanin has high affinities for various molecules that may bind to them 7, and covalently bonded polysaccharides have been detected in melanin that survived enzymatic and acid treatments.