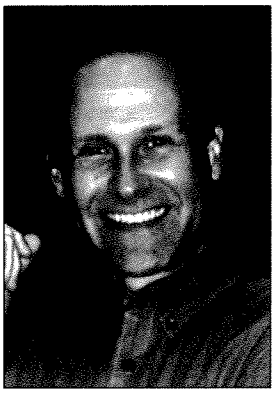

Immunostimulatory Activity of Echinacea species:

An Interview with David Pasco, Ph.D.

by Glinda Watts, RH (AHG)

David S. Pasco, Ph.D. is the Assistant Director of the National Center for Natural Product Research at the University of Mississippi and is principal investigator for two grants from NIH/NCCAM to study melanin and its immuno-stimulatory activity within three Echinacea species; *E. angustifolia, E. pupurea, and E. pallida*. The research described here has been published in *International Immunopharmacology 5* (2005) 637-647.

Watts: Was your NIH grant specifically to study melanin?

Pasco: Yes, specifically the melanin in echinacea. We wanted to study echinacea source material from all over the country to see how much variation in melanin activity we could find. We analyzed many different echinacea products looking for melanin. So far, melanin is pretty much non-existent in tinctures. We are also studying the agricultural parameters that are important for promoting optimal melanin content of echinacea plants and activity as well as determining the structural features of melanin that are important for its ability to activate monocytes/macrophages.

Watts: Why the focus on melanin? It's never mentioned as a constituent of echinacea in any reference that I've ever seen.

Pasco: The understanding of melanin as an immuno-stimulatory compound is a new discovery that we made based on previous research funded by NIH that started back in 2000. The first grant was to look at immuno-stimulatory activity within echinacea. We had the evidence that there was something in the plant that was really, really, potent that could not be accounted for by any of the other compounds that were thought to contribute to echinacea's activity. It wasn't the polysaccharides, it wasn't the isobutylamides, or the echinosides or cichoric acids or any of those things so we knew then that there was something different there and that was the basis for NIH giving us the grant. During the course of the two-year grant we found that it was the melanin that was responsible for this immuno-stimulatory activity.

Watts: I understand you also discovered melanin in other plants.

Pasco: Yes, ginseng has even more active melanin than echinacea. Other melanin notable plants include black walnut hull, alfalfa sprouts, green tea, horsetail, astragalus, and chamomile. Ephedra has none, St. John's wort has very little, black tea has 5,000 times less melanin than green tea. All vegetables have 1000 to 10,000 times less melanin than the most active herb.

Watts: What is the role of melanin in plants?

Pasco: We really don't know. It probably offers some UV protection just like in people but it may also be used as a protectant against microbial infection. In some of our research we treated alfalfa sprouts — which are very high in melanin — with chitin, which is an elicitor of plant defense mechanisms because it mimics fungus. The plants detect the chitin and think that they're being attacked by fungus. Within 48 hours after treating the plant with chitin, the immuno-stimulatory activity of the melanin increased 25 times.

Watts: Have you analyzed commercial echinacea extracts for melanin content?

Pasco: Tinctures contain almost no melanin. They contain the isobutylamides and that would give an anti-inflammatory effect. Even the fresh-pressed juice

contains only minimal amounts of melanin. Plus melanin is very insoluble, so even if you extract some out, it's going to start settling and companies filter extracts to make them clear and attractive to the consumer, but this filters all the activity out.

Watts: Describe the experiment that first gave you a clue to the existence of an unknown active immunostimulant within echinacea.

Pasco: We started out by growing *Echinacea angustifolia* under aseptic conditions in vitro. The echinacea was then extracted with the following solvents: water at 70 degrees C, methanol, ethanol, dichloromethane, hexane, ethyl acetate. The human monocyte cell line THP-1 was treated with these extracts at a concentration of 75 mcgs/ml. The cells were also treated with finely ground echinacea material (20 mcgs/ml) that had not been extracted with any solvent. The positive control was LPS at 10 mcg/ml and activation by this agent was termed 100%. Ground echinacea gave 50% activation, water 24%, and the other solvent extracts did not induce significant activation. Finely ground plant material from seven other botanicals (run at 20mcg/ml) did not induce any activation. So at that point we knew that there was something different about the composition of the echinacea plant material as compared with the other seven botanicals and that the solvents tested were not capable of extracting the majority of this activity. Since the solvents tested could extract all known echinacea components we knew there was something unknown responsible for this high activity.

Watts: Would you please explain what the control substance LPS is?

Pasco: LPS is the lipopolysaccharide from the membrane of *E. coli*. It is a benchmark for activation of macrophages that is widely used in research. The more active melanins are just about as active as LPS. When you compare activation of most polysaccharides it takes 10-100 times more polysaccharides compared to the LPS, whereas the melanin seems to be about equal in activity to the LPS. From our calculations the amount of activity contributed by the polysaccharides in echinacea is considerably less than the melanin. The polysaccharides are so weak and there is so little of them in there that their effects may be negligible.

Watts: So you have observed more than one type of melanin?

Pasco: Yes, some structural features seem to be responsible for the immune activity. This is what we are trying to find out in a subsequent grant.

Watts: After you applied the various extracts of echinacea to the monocytes and noted no immunostimulatory activity, you then sprinkled ground echinacea powder. What happened?

Pasco: When we sprinkled ground echinacea into the culture dish we got tremendous activation of the monocytes. For the control, we sprinkled ground plant material from other herbs, which showed no activity. This let us know that it wasn't simply the dried herb causing the activation, but that there was something compositionally different with the echinacea that was causing the activity. So once we knew that there was all this activation from the echinacea, yet all the solvents weren't capable of pulling it out, we said 'well, its worthwhile trying every solvent in existence to see which one would extract it'. The first solvent that we found that was capable of extracting the melanin was 90% aqueous phenol. However we have also subsequently found that melanin can be extracted by non-toxic methods.

Watts: Was water an effective solvent?

Pasco: Water is active, but to put things in perspective, even though water is quite active, when you do a hot water extraction you are only extracting 1% of the total melanin in echinacea.

Watts: Were you able to observe how echinacea goes about activating immunity in vivo?

Pasco: We used a mouse model to test the effect of large molecular weight immunostimulants that would be relevant to oral ingestion. We extracted the melanin from the plant material, purified it, mixed it with mouse chow, pressed it back into pellets and fed it to the mice. In these experiments various immune parameters were compared between mice that ate food alone vs. food with melanin. The melanin-eating mice got 10-25 mgs. of melanin a day. If you do this for 4 days, the mice will have higher IgA production in the Peyer's patches, lymphatic tissue

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which protects the GI tract from foreign bacteria. The IgA then gets secreted and coats everything passing through and prevents anything from clinging to the intestinal wall. Il-6, one of the cytokines responsible for enhancing IgA production, is increased 2-3 times from these cells. Production of interferon from spleen cells in these melanin-treated mice increases 5-6 times after 4 days of eating melanin-enhanced food.

Our *in vitro* experiments showed that melanin activates monocytes through a toll-like receptor 2 dependent pathway, a receptor normally used to recognize bacterial and fungal components in the GI tract. Melanin mimics these pathogens. When bacteria

Echinacea purpurea (purple coneflower)

and fungi enter your GI tract, they're not soluble; they're particles. And yet the immune system knows they're there. Your immune system is set up in such a way in your GI tract that it doesn't matter if something is soluble or how big it is. In fact, the bigger the particles are, the more the immune system responds; the immune cells are more readily able to detect intestinal contents. They sift through the intestinal contents constantly to see if there is anything pathogenic, and they encounter the melanin and it's perceived as bacteria or fungus and the immune cells get activated. The activation remains within the gut however and does not spill over systematically. This is thought to prime the immune system. A good example of this is found by looking at germ-free animals developed for research. Germ free animals have no intestinal flora; these mice immune systems are basically non-existent. Spleen size is about a third, lymph nodes along the intestines are involuted by about a third, with hardly any cells within. It's difficult to get these mice to clear bacteria...they are really compromised with hardly any antibody response. This shows how important intestinal flora is not only for your gut mucosa but every mucosal surface: GI, respiratory tract, urinary tract — they're all linked by the mucosal immune system. So when you influence the mucosal immune system in your gut, your nose, esophagus, bladder — anywhere in the body — every mucosal system gets the benefit. In one study that I know of, mice were given antibiotics to wipe out their intestinal flora. The intestinal flora were re-introduced in one group of mice and in the other group of mice they put some flora plus an over abundance of Candida and then measured the response of those two groups of mice to an allergen. The mice that had normal intestinal flora had almost no response to the allergen. The mice that had the over abundance of Candida had just a huge full-blown reaction. The field of mucosal immunology is really exploding right now.

Previously, with botanicals that have been traditionally used to enhance immune function, the thinking was that the constituents had to enter the blood stream to affect the immune system. That's totally changed now. What we're finding is that you have polysaccharides in these herbs and melanin too, and they don't get absorbed but the immune system detects them and thinks it's being invaded by pathogens and your immune system gets toned up because of that.

Watts: Are you doing any research that might identify other constituents in echinacea as being immuno-stimulatory?

Pasco: We are in the process of writing another paper where we compare the activities of echinacea

polysaccharides with echinacea melanin both *in vitro* and in the mouse model we used in the original melanin research. What we found was that the polysaccharides from echinacea worked through toll-like receptor 4, whereas the melanin worked through toll-like receptor 2. There are 11 different toll-like receptors. Melanin is substantially more active both in vitro for monocyte activation (where melanin is 100 times more active for IL-1 beta production) and in the mouse model for interferon gamma production from spleen cells (where melanin is at least 20 times more potent than polysaccharides).

The polysaccharide experiments that were done initially showed immune stimulation but these studies injected the echinacea either IP or IV and the polysaccharides were purified as well. The polysaccharides were extracted from a pound of plant material and then injected into a mouse. That's totally unrelated to the way echinacea has been used traditionally — it's always been consumed. So these studies showed activity but not relevant to the normal dose or usual means of delivery.

Watts: Are you saying that this research demonstrates that melanin is THE sole constituent responsible for immune stimulation in echinacea?

Pasco: All we are saying is that melanin is a previously overlooked constituent of echinacea that has potent immunostimulatory activity. We are also saying that in the experimental systems we have used that the polysaccharides exhibit substantially less activity than the melanin.