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פרופ' דניאל זייפמן
נשיא
Prof. Daniel Zajfman
President

January 18, 2010

Mr. Benedetto de Benedetti
Mrs. Elisabetta Salza
De Benedetti Foundation-Cherasco 1547
Via Santa Teresa 12
Torino 10123
Italy

Dear Benedetto and Elisabetta,

I am writing to convey my thanks to you for the generous contribution the De Benedetti Foundation has been making to scientific research conducted in the framework of the Torino-Weizmann Exchange Program.

It is very gratifying to see us now in the third year of activity, with support this year going to a project between Prof. Bershadsky and Prof. Defilippi. I am very pleased that the program is well on track and the network of scientific collaborations is widening.

We are all very grateful that you have instigated and, in a sense, have acted as midwife, to the two earlier projects. The first collaboration between Prof. Emilio Hirsch from the University of Torino and the Institute's Prof. Ronen Alon focused on the involvement of an enzyme family in the process through which T cells cross the blood-vessel walls to get to the point where they are needed. In their work, a novel mode of lymphocyte movement was identified, in which the lymphocytes send out tiny "legs" and use them to sense the environment and move toward infected cells.

The second year's project between Dr. Cinzia M. Bertea from Torino's CEBIOVEM, and Dr. Asaph Aharoni from the Institute's Department of Plant Sciences, explored, on the molecular level, the mechanism that regulates an active chemical compound in a plant that has historical medicinal and ceremonial uses.

I am enclosing a report in hard copy and DVD on the two projects, which you are free to use as you see fit to promote the program among additional potential supporters.

I look forward to welcoming you back on campus soon. With warm regards and all good wishes for the year ahead,

Sincerely,

Daniel Zajfman

DZ/sg

cc: Prof. Israel Bar-Joseph, Vice President, Resource Development and Public Affairs
Prof. Lia Addadi, Committee Chair, Torino-Weizmann Exchange Program
Kelly Avidan, Secretary of the Association and Director, Dept. of Resource Development
Gila Shmueli, Special Projects, Resource Development and Public Affairs

The Torino-Weizmann Exchange Program

Report to the
De Benedetti Foundation - Cherasco 1547

January 2010

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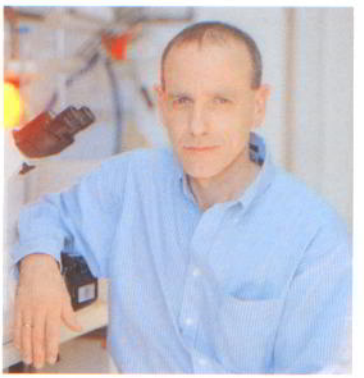
WEIZMANN INSTITUTE OF SCIENCE

Targeting Inflammatory Lymphocytes

Profs. Ronen Alon and Emilio Hirsch



Prof. Ronen Alon

Prof. Emilio Hirsch

The immune system guards the body against infection. Among its components are T cells, which are white blood cells that play a central role in the orchestration of immune responses against pathogens. They employ elaborate signaling processes, involving various chemical messengers.

Once they have identified an invader, T cells generate specific responses to eliminate pathogens or pathogen-infected cells. They circulate in the blood, crawling swiftly along the lining of the blood vessel, gripping it tightly to avoid being swept away in the blood flow, all the while searching for temporary "road signs" and "exit signals" made of special molecules, that let them know where to cross the blood vessel barrier, so they can get to the inflamed or infected tissue.

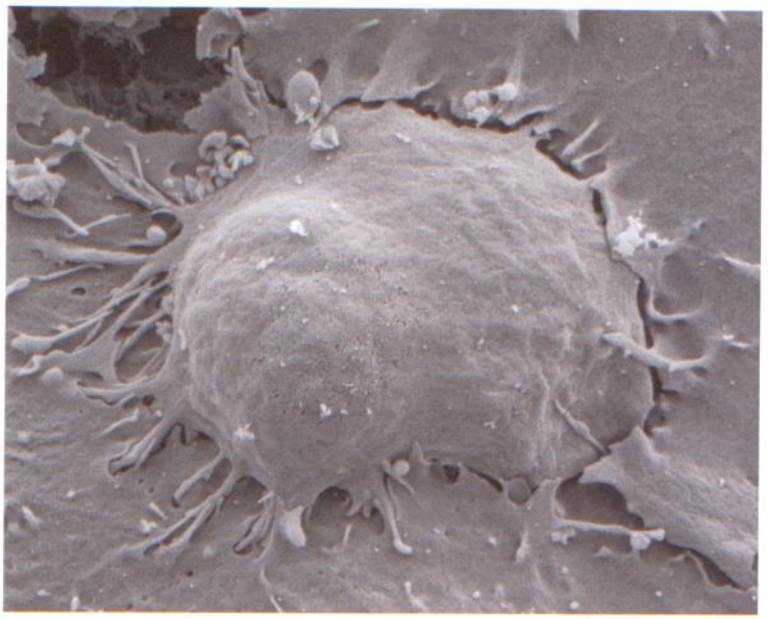
But how do they get to their specific target and cross the blood-vessel barrier? Building on their distinct expertise fields, Prof. Ronen Alon at the Weizmann Institute's Department of Immunology and Prof. Emilio Hirsch at the University of Torino's Department of Genetics initiated a collaboration aimed at exploring the involvement of an enzyme family in this process. The Phosphoinositide 3-kinases (PI3Ks) family of enzymes are involved in cellular functions such as cell growth, proliferation, directionality of the cell, motility, survival, and intracellular trafficking, which are, in turn, play a part in cancer and many other diseases. They have a crucial role in a variety of communication chains in the immune system, to which movement of cells is key.

Their collaborative efforts centered on the role of PI3Ks in regulating the activity of a sub-group of T cells called effector T cells. This sub-group specializes in entering sites of infection and inflammation and killing the infected cells. The collaborators decided to examine whether PI3Ks also have a facilitating role in T-cells' crossing of blood vessel walls. Tissue samples supplied by the Hirsch lab, from mice whose PI3K genes were rendered inoperative, or "knocked out," were used in the Alon lab to follow the movement of effector T-cells "programmed" to identify a specific antigen.

While some of the PI3K family members were not found to be directly involved in this initial stage of T cell movement out of blood vessels, this study succeeded in shedding light on a novel mode of T cell movement along the cells lining the blood vessel walls. This type of T cells movement occurs close to the point at which they cross the blood vessel wall into the infected site beyond it.

Contrary to previous notions, T-cells were found to use tiny "legs" to advance toward inflamed cells. Termed filopodia, these "legs" are tiny protrusions, no more than a micron (1 millionth of a meter) in length and tens of them in each T cell, that attach and detach in sequence within seconds, providing T cells with a good grip of the blood vessel's sides and enabling their rapid movement. Scanning and transmission electron microscopy images showed that upon attaching to the blood vessel wall, the filopodia "dig" themselves into the blood vessel wall, pressing down on its surface. From this up-close view, the scientists believe that filopodia are tri-functional: used for gripping, moving, and sensing distress signals from the damaged tissue.

In the future, the two research groups plan to explore potential roles of specific members of the PI3Ks family in the navigation of effector lymphocytes into tissues, once these cells have crossed the blood vessels at sites of inflammation and infection.



Effector lymphocytes attached and spread on an inflamed endothelial cell. The sample was fixed and imaged by scanning electron microscopy. Note the numerous invasive filopodia sent out underneath the lymphocyte and penetrating deep into the thin endothelial cell.

The Molecular Characterization of Boswellia, and the Biosynthesis of a Novel Pharmacological Agent from an Ancient Drug

Drs. Asaph Aharoni and Cinzia M. Bertea

Dr. Asaph Aharoni

Dr. Cinzia M. Bertea

Frankincense (also called "olibanum" and "lebona"), an aromatic resin obtained from trees of the genus Boswellia, has great commercial and cultural value. Beyond its main cultural and religious uses, its medicinal properties are also widely recognized, mainly for the treatment of inflammatory conditions, some cancerous diseases, wound healing, and as an antimicrobial agent.

Until recently, investigations of Boswellia focused on the resin's effects on the immune system, and boswellic acids were considered to be the main, if not the only, active ingredients of the resin. Only recently, the resin was screened for its biologically active constituents; the results point to incensole acetate (IA) and its derivatives as the major active constituents. These compounds, members of the cembrenoid diterpene family, exert intriguing biological activities, and may prove to be of considerable pharmacological value.

Separate research in mice identified the compound IA and its derivatives to be responsible, at least in part, for its anti-inflammatory effects; to have a robust neuroprotective effect following head trauma; and to exert anti-depressive and anxiolytic effects (suggesting a pharmacological explanation for the common religious and ceremonial uses of the resin throughout the centuries). These, and additional findings, led scientists to believe that IA and its derivatives, cembrenoid diterpenes, play significant roles in the effects that Boswellia resin exhibits on biological systems.

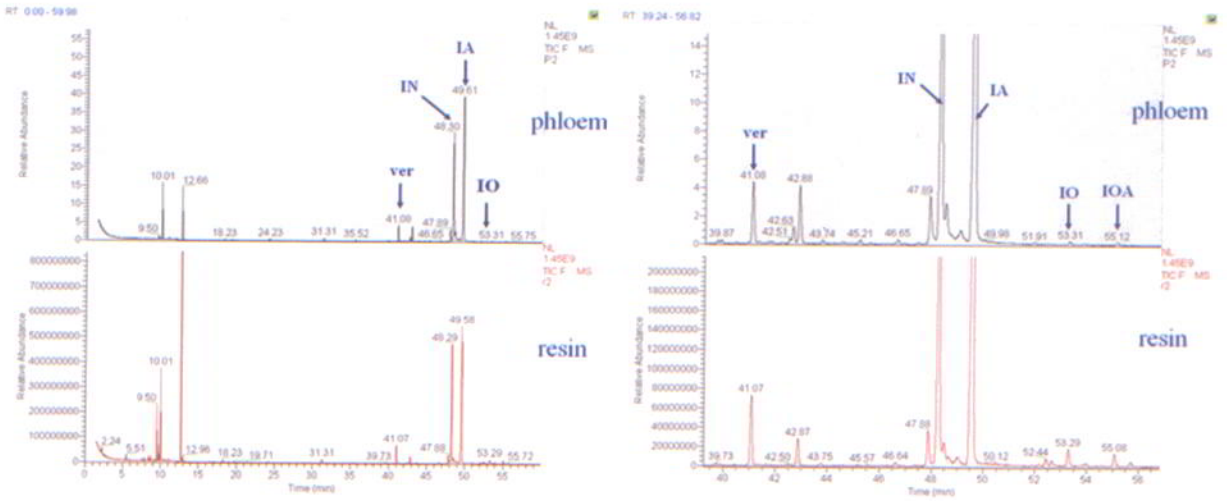
Despite the benign effects of these molecules, their use as pharmacological agents is limited by the fact that their chemical synthesis is not readily feasible, leaving the plant as the only available source. By elucidating their biosynthetic pathway, scientists may be able to effect their synthesis or metabolic engineering in a different plant or bacteria.

Supported by the De Benedetti Foundation, Dr. Asaph Aharoni of the Weizmann Institute's Department of Plant Sciences and Dr. Cinzia M. Bertea from the University of Torino's Department of Plant Biology and Centro di Eccellenza per la Biosensoristica Vegetale e Microbica (CEBIOVEM) initiated a study that aims at elucidating the biosynthesis of IA in the Boswellia resin, using combined biochemical and molecular biological approaches.

This work was performed as a close collaboration between the two researchers. Dr. Bertea and the Italian research group have wide expertise in molecular biology and biochemistry of plant secondary or specialized metabolism (i.e., natural products) and medicinal plants. The Weizmann research group is studying the regulation of metabolic pathways in plants, particularly secondary metabolism. The group has extensive expertise in the analysis of secondary metabolites and functional analysis of genes associated with it. The postdoctoral fellow working on the project, Dr. Arieh Moussaieff, has a vast experience in the chemistry and the pharmacology of Boswellia resin, and has previously made important contributions to this topic.

The collaboration between the two research groups in the course of this one-year project, is exemplified by Dr. Bertea's month-long visit at the Weizmann Institute, where she worked on the cloning of the putative diterpene synthase related to IA biosynthesis. Dr. Moussaieff presented the results of this work in the international meeting on "Metabolism, Metabolomics and Metabolic Engineering in Plants to Increase Crop Productivity and Nutritional Value" (Israel, November 2009).

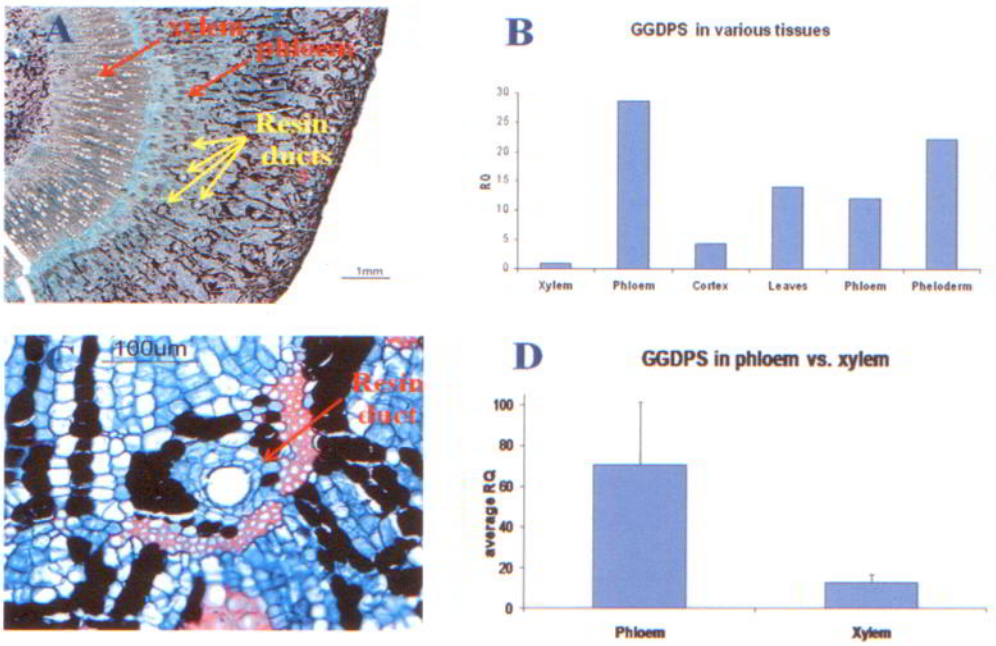
They first analyzed the chemical profiles of the Boswellia resin and other plant tissues, by GC-MS, or gas chromatography-mass spectrometry, a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Although several studies were performed on the chemical composition of the resin, this was the first comprehensive analysis of the tree (see results below).



GC-MS results: incensole and its derivatives accumulate in the resin ducts located in the phloem. Ver = verticillol; IN = incensole; IA = incensole acetate; IO = incensole oxide; IOA = incensole oxide acetate.

The phloem is the living tissue that carries organic nutrients, particularly sucrose, to all parts of the vascular plants where they are needed. The researchers undertook gene cloning, synthesis, and amplification in the phloem of the Boswellia plant's bark and branches and then compared it to other tissues.

To localize the site of synthesis of the IA cembrenoid diterpenes derivatives in the Boswellia plant, the researchers cloned the geranylgeranyl diphosphate synthase (GGDPS) gene, which encodes the enzyme that performs the first irreversible step in the synthesis of the precursor (geranylgeranyl diphosphate, or GGPP). Using a gene amplification method (quantitative real-time PCR), they detected higher expression of GGDPS in the phloem of the bark and branches in comparison to the other tissues (see B and D in the figure below). This was in fact the first time that genes have been isolated from this Biblical plant species.



Boswellia resin ducts are located in the phloem of the stem and branches, as depicted in a histological safranin\fast green staining (A, C). The GGDPS gene showed higher expression levels in the phloem in comparison to the other tissues, suggesting that diterpenoid production including IA) is major in the phloem tissue (B, D). The possible localization of terpenoid production in the phloem was confirmed by the means of microscopic analyses. Thin cross sections of Boswellia bark and branches treated with Safranin and Fast Green showed the distribution of resin ducts in the phloem (A and C).

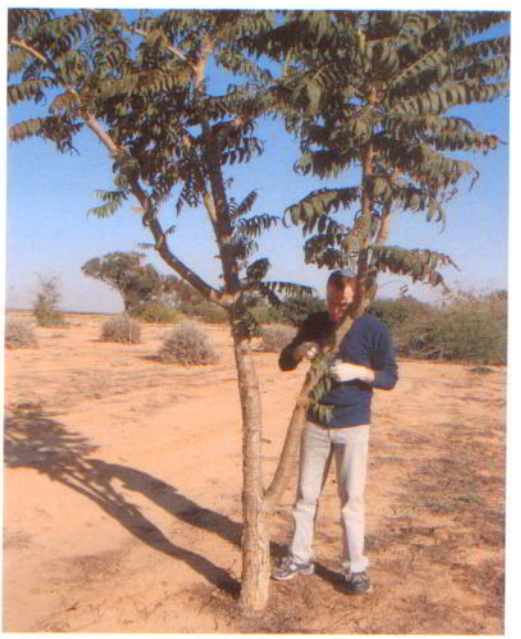
Drs. Aharoni and Bertea then turned to isolating the multiple genes related to the biosynthesis of IA and its derivatives. Using molecular genetic methods, they were able to clone a diterpene synthase-like gene that showed a relatively high expression in the phloem, corroborating the findings on the site of resin production, and confirming the working hypothesis on the concomitant site of

derivative production. This gene also demonstrated a high homology, or resemblance, to another enzyme, casbene synthase.

They subsequently used new generation sequencing, a cutting-edge technology that allows the comprehensive analysis of the transcriptome (full set of all RNA molecules produced in one cell or a cell population that reflects the genes that are being actively expressed at any given time) of Boswellia. Further bioinformatic analyses led to the identification of 25 overlapping DNA segments related to terpenoid biosynthesis. Of these, four are homologous to casbene synthase and are highly expressed in the phloem of the plant when compared to expression in the xylem tissue.

Cloning the full length genes and their functional analysis (enzyme activity assays) through heterologous expression in Escherichia coli is currently underway.

The recent insights into the production of the Boswellia resin and its major active constituents provided by Dr. Aharoni and Dr. Bertea are important steps towards the elucidating the biosynthetic pathways of Boswellia resin components, including IA and its derivatives. The findings may also pave the way to a feasible biosynthetic production of novel prototype potential drugs, which may potentially target unaddressed pharmaceutical needs of large segments of the population. The identification of a new prototype drug with beneficial effects in fields of considerable current interest may further open these fields to the discovery of novel drugs for the treatment of diseases that pose unanswered challenges.



The postdoctoral fellow Dr. Arieh Moussaieff samples the resin of a Boswellia tree growing in the south of Israel