Lymphology 21 (1988) 83-85

EDITORIAL

PUTTING THE IMMUNE SYSTEM BACK IN THE BODY

I have been culturing normal human blood mononuclear cells for years *in vitro* in media supplemented with 10-20% fetal calf serum or autologous human serum. Normal cells respond actively to mitogens and alloantigens, but cultured cells from cancer patients are much less responsive. The conclusions seem clear: lymphocytes of cancer patients are defective and/or the serum suppressive, an interpretation well accepted in clinical practice. However, some doubts began to emerge in my mind as to whether the interpretation of these findings is entirely valid. Is it possible we create misleading shortcuts in reasoning from the test tube to the whole patient? Can what we see in blood cell culture be legitimately extrapolated to circulating blood? For example, circulating cells are suspended in 100% serum (plasma), and the statistical probability of their coming into contact with accessory cells presenting mitogens or alloantigens is practically nil. On the other hand, the concentration of mitogen used in culture would be highly toxic for the living organism. Thus, to approximate *in vitro* conditions to the sick patient, I cultured whole blood, and also mononuclear cells, in whole serum. Surprisingly, cell responsiveness turned out to be very poor! In other words, white blood cells taken out of their normal environment reacted differently. Is it because there is little or no cell-to-cell contact in whole blood because of mechanical separation by innumerable erythrocytes, or are cytotoxins lacking from degraded neutrophils, or is PHA inactivated or do serum factors inhibit outbursts of free reactivity?

This simple example illustrates how easily and perhaps misguidedly we extrapolate results obtained from *in vitro* testing to the whole organism. Although the laboratory data may often prove useful, it also may be misleading with serious consequences on treatment regimens. Sensitized by the discrepant findings with blood culture, I started to investigate how accurately *in vitro* immunologic testing and models mimic the *in vivo* situation or conversely, how far these laboratory phenomena are removed from physiological realities. To my dismay, I found a multitude of examples demonstrating a notable dichotomy between the *in vitro* and *in vivo* circumstance.

Before an immune reaction can begin, an antigen in the form of bacteria, virus or allogenic transplanted cell encounters a host immune cell. The site where this contact takes place is most likely in lymph node, spleen, or bone marrow, and not in circulating blood where cells travel swiftly, the physical distance between them prevents effective interaction, and where plasma factors coat cell surface and inhibit reactivity. Nonetheless, for testing immune reactions we almost exclusively use blood cells because other sources of materials are clinically unavailable. We should remem-

ber, however, that circulating blood cells are not fully representative of the node and spleen cell populations most active in the immune process, and that they are relatively inert in plasma.

In order to reach foreign antigen deposited in the tissue, immune cells must traverse the highly discriminating capillary wall, then migrate to the site of the antigen. Motility, mobility, directional migration--how differently these phenomena look *in vitro* and *in vivo*! The *in vitro* migrational tests tell us about the potency of chemical attractants and compulsory directional regulation of cell motility. *In vivo*, however, it is the cell's own discriminative ability which governs the leucocyte drive toward "non-self" molecules.

In vivo, the antigen itself is carried by host cells to reach organized lymphoid tissue. A harmonious cooperation between cells of different specializations is thereby initiated. The local humoral environment (lymphokines) facilitates the development of the reaction. There are no *in vitro* counterparts to mimic fully this intercellular cooperation.

In vivo, the same immune recognition is a prerequisite for initiation of immune reaction and for induction of specific tolerance. The issue is not only which are the recognition molecules (most studies *in vitro* are oriented in this fashion) but also, the much less extensively studied question of which cell subsets first recognize "non-self" and what stimulates them to distinguish "self" from "non-self."

Whereas allogenic lymphocytes grown in mixed culture with incompatible recipient blood, lymph node, or spleen cells remain viable after 6 hours in the test tube and become activated, lymphocytes from the same donor infused intravenously are destroyed within 6 hours in a previously non-sensitized recipient's lymphoid organs. This latter phenomenon is called "allogenic lymphocyte cytotoxicity-ALC", and reflects a notable discrepancy between *in vitro* and *in vivo* findings.

The signal for mobilization of lymphocytes in case of tissue penetration by foreign antigens (e.g., wound infection) is integrally linked with the reaction of the neurohormonal system and specifically cortisol secretion. *In vitro* treatment of lymphocytes with cortisone affects their mobility and responsiveness to mitogens. *In vivo* administration of cortisone causes rapid depopulation of blood, lymph nodes, and spleen of lymphocytes, and promotes their accumulation in the bone marrow. If the lymphocytes truly as suggested by *in vitro* experiments lose mobility, how is it they migrate *in vivo* so quickly to the bone marrow with the same stimulus?

Activation of immune cells involves a cascade of cellular interactions and presence of various subpopulations. In the mixed allogenic lymphocyte culture the proportions of stimulating and responding cells do not correspond to, and do not even approximate the ratios *in vivo*. In the whole body, there is a continuous recruitment and replenishment of cells at the site of antigen deposition, such that quantitative cellular requirements are being met according to the kinetics of the reactive process. Not only is this rarely considered with *in vitro* experiments, but the *in vivo* process lasts considerably longer than can be simulated *in vitro*.

Activating blood mononuclear cells with interleukin-2 leads to a rise in lymphokine-activated killers (LAK). When these mononuclear cells are injected intravenously they lodge most prominently in the liver; but does the *in vivo* activation with IL2 proceed in a similar way? There seem to be major differences in body-organ distribution of *in vitro* and *in vivo* stimulated lymphocytes.

I have cited these examples in order to illustrate how wide the biologic gap

may be between what we observe *in vitro* and that *in vivo*, and how spectacular and fitting to our expectations are the *in vitro* findings, and, yet, how unexpected, complex, and puzzling (but surely closer to the truth) are the findings from whole-body investigations. Indeed, the dichotomy of findings happens frequently. But, because the complex jigsaw puzzle of the living organism is near impossible to reconstruct, the *in vitro* studies with well-organized and seemingly convincing methodologies are etched powerfully in books and journals, and win prizes and prestige. It is not my intent to deprecate the value of these *in vitro* studies enriching basic sciences, and in some instances, revolutionizing understanding of the molecular fabric and organization of the immune system. Further, it would be foolhardy to deny that molecular biology needs the test tube. But as we are dazzled by the spectacular achievements in understanding the molecular structure of receptors, metabolic pathways, and drowned in a sea of new lymphokines raised in culture wells, we should not lose contact with the living organism. Involvement in *in vitro* investigations consumes much of our energy and working time, and often leaves too little time for physiologic meditations. It is so much easier to manipulate biological material in the Petri dish than to penetrate the complex machinery of the intact host. The body of knowledge in basic immunology has become so vast that the time has arrived to adjust the pendulum back toward *in vivo* physiological studies. Knowledge of how the immune system interacts and integrates is still rudimentary, and unless we develop well-controlled experimental models we only describe qualitative phenomena. We still lack methods for quantifying the results of manipulating the immune system.

The future of immunology should properly be an era of immunophysiology with special emphasis on immunoregulation. Major thrusts of development of *in vivo* research can be delineated by four C's (Communication, Cooperation, Contact, and Control).

COMMUNICATION within the immune system and with other life-support systems:

1. Cell traffic kinetics--mechanism of mobilization and recruitment, physiological extravasation and recognition of site of origin (homing), *in vivo* recognition of targets, functional structure of lymphoid tissue (leucocytes, lymph nodes, spleen, bone marrow), lymph composition and movement.

2. Communication and cooperation with the nervous and endocrine systems--evolution and ontogeny, anatomical and biochemical links.

COOPERATION between lymphoid cells: environment for maturation and specialization, mechanism of cooperation within different lymphoid organs, lymphokines (local or body-wide) and synaptic-regulating hormones.

CONTACT of lymphoid and non-lymphoid (parenchymatous) cells: "immune surveillance" against invaders and mutants, regulation of "self."

CONTROL of *in vivo* immune events: manipulation with natural and synthetic regulatory molecules and drugs.

Immunity properly belongs to the whole body. Its anatomical components are lymph, lymphatics, lymph nodes, spleen, bone marrow, and circulating white cells. Let us study them in the context of living interrelationships, while making use of the enormous volume of "test tube" knowledge accumulated over the past several decades.

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