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Issue Highlights for May 2003

Mycobacterial infections in HIV-infected patients: A rôle for phagocyte dysfunction

Both typical and atypical mycobacterial infections cause a great deal of morbidity in patients with HIV infection. In part, this is caused by a general debility of patients with frank AIDS, who for nutritional and other reasons may be more broadly susceptible. In part, this is because of altered cellular immunity, the pathophysiologic hallmark of AIDS. There have been some observations, however, suggesting that monocyte and granulocyte function is abnormal in HIV-infected patients, and that this may also be an important determinant of risk. It's been unclear to date whether that phagocyte dysfunction is the result of abnormal levels of cytokines, abnormal levels of immunoproteins such as immunoglobulin and complement components, or a defect residing in the cells themselves.

To try to parse out the relative importance of the cells and their milieu, *Dr Daniel Kaul and colleagues* from the University of Michigan harvested neutrophils and monocytes from normal subjects and from HIV-infected subjects, stratifying the latter according to the CD-4 lymphocyte count. They tested the ability of these cells to impair the growth of *Mycobacterium bovis* organisms that were added to the cell preparations at a ration of 5 organisms per cell. After a 24-hour incubation, the cells were lysed (to release any organisms that had been phagocytosed but not killed, or that had actually infected the cells), and the organisms were transferred into a quantitative cultures system to determine their growth index.

As expected, incubation with normal phagocytes led to a lower growth index when the *Mycobacteria* were subsequently cultured. When the phagocytes had been obtained from HIV-infected patients, however, the inhibition of growth index was less striking. The effect associated with monocytes was 81% greater, and that associated with neutrophils was 69% greater, if the cells had come from normal donors. The authors found that the inhibitory effect was better preserved when phagocytes were obtained from HIV-infected patients who were clinically well and whose CD-4 counts were preserved.

Attempts to correct the defect by the addition of GM-CSF, IL-2, or IL-8 were not successful. The authors suggest that there is an intrinsic phagocyte functional defect in HIV-infected patients, and that this might be a contributor to risk of mycobacterial infection. Just how such a defect arises is not at all certain, but it does not correct in the short term by adding additional cytokines.

This paper may be found on page 330.

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Ethics forum: Cultural issues in research oversight

Research regulatory oversight in the US grew largely out of concern for and response to scandal; it has been heavily conditioned by the specific scandals (and near-misses) that have occurred in this country, and its structure has developed to reflect our values and concerns. Particularly important direction was given to this process by the April 1979 report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research ("the Belmont Report"). That report—itself spurred by revelations about a US Public Health Service study of the natural history of untreated syphilis—stressed three ethical principles: beneficence, justice, and respect for persons (and their autonomy).

The Belmont Report and the regulations drafted to reflect it provide valuable guidance for the proper conduct of research with human subjects, as long as both the researchers and subjects come from the same cultural background as that underpinning the report. Increasingly commonly, that is not the case. International research is becoming more common in general; more specifically, drug and device trials conducted in other countries are being designed such that their findings may be presented for licensure applications in the US. An attempt has been made to make such international research more predictably acceptable: the International Conference on Harmonisation has drafted guidelines to make requirements uniform from country to country for research intended to support a drug licensing application. The ICH guidelines are largely based on the US Food and Drug Administration regulations (at 21 CFR §50, 21 CFR §56, and 21 CFR §312), and thus reflect the societal premises behind the Belmont Report.

At first blush, this would appear to be a good thing. It makes it hard for an unscrupulous drug developer to do the most dangerous trials in countries where the regulations might not be as stringent, then use ethically questionable findings to achieve drug licensure in countries with strict oversight requirements. But this benefit is not without its rough edges. How do you apply the US norms for acceptable risk to a country where far less medical care is available and daily life carries higher risk? How do you apply the US norm for information and documentation of consent to a population where many of the subjects will not be able to read or write? How do you apply the US norm for individual informed consent to a context where the smallest unit of decisional authority is something other than an individual, such as a family? Indeed, how do you decide when the research is exploitative?

Dr N. Yasemin Oguz, of the Department of Medical Ethics at the Ankara University, reviews for us this month the experience of trying to adopt an ICH-compliant approach to human subjects' research in Turkey, where several major cultural differences strain the applicability of international "norms." Her essay may be found on page 292.

Shear stress and thrombin generation

Under shear conditions approximating those in small arteries, platelets become activated. The activation is characterized by increased binding of von Willebrand factor (vWF) to glycoprotein Ib (GP-Ib) and to GP IIb-IIIa (also known as integrin $\alpha_{IIb}\beta_3$). When these adhesive receptors are occupied, the platelet undergoes conformational changes—such as increasing the surface exposure of anionic phospholipid—that make it a better supporter of plasma coagulation. A study linking shear stress to plasma coagulation via the platelet is presented in this month's issue by *Dr Jeffrey F. W. Keuren and his colleagues* from the University of Maastricht, Netherlands (see page 350).

Platelet-rich plasma was placed in a warm cuvette in which a rotating cylinder could provide a consistent shear stress. Calcium was added, and thrombin generation was monitored continuously via a fluorescent detector target.

Abciximab—an antagonist of integrin $\alpha_{IIb}\beta_3$—reduced the peak of thrombin formation, especially at the higher shear rates examined. This was apparently caused by the known binding characteristics of abciximab, as the drug did not lower the peak of thrombin formation in stirred PRP from patients with Glanzmann's thrombaesthenia (whose platelets lack $\alpha_{IIb}\beta_3$). Moreover, in PRP from control subjects, antibodies specifically directed against vWF-binding epitopes on GP Ibα

reduced the thrombin formation at high (but not at low) stirring rates. The two strategies combined (abciximab and anti-GP Ib antibody) more strongly to inhibit thrombin formation at high shear rates.

The authors conclude that thrombin formation and coagulation in stirred PRP is to a large extent dependent on platelet activation, with vWF adhesion to integrin $\alpha_{IIb}\beta_3$ and—in a shear-dependent way—on GP Ib.

Mesenchymal stem cells from the pancreas

The ability to isolate stem cells of various sorts from different types of tissue has been of great scientific and political interest in recent years. It is becoming clear that many tissues harbor cells that are not terminally differentiated; another chapter in this ongoing story is presented this month by *Dr Ying Hu and associates* from the Chinese Academy of Medical Science & Peking Union Medical College, Tianjin and Beijing, P. R. China. These investigators have been studying mesenchymal stem cells, and now report their isolation from fetal pancreas.

As described beginning on page 342, pancreatic tissue was obtained from spontaneously aborted fetuses. Mononuclear cells were separated on a density cushion, then depleted of $CD45^+$ and glycophorin-A^+ ($GlyA^+$) cells by means of micromagnetic bead sorting. These were then plated onto fibronectin-coated wells and the colonies were harvested.

The resultant cells were rapidly proliferating, and could be carried through roughly 30 passages without morphologic change. They were immunophenotypically CD44, CD29, and CD13 positive and negative for CD34 and HLA-DR. Immunohistologically, they were recognized by antibodies against collagen I and III but not by antibodies against von Willebrand factor. During the log phase of growth, these cells had a doubling time of about 30 hours, but only a small population of cells was actively engaged in proliferation (S+G2+M=3.55%). The claim that they were actually stem cells was supported by the ability, under different culture conditions, to make them differentiate into cells of osteogenic, chondrogenic, and adipogenic lineages.

Ferritin and exposure to oxidative stress

We're used to thinking of serum ferritin as a useful marker of iron stores, and its occasional elevation in inflammatory states is a nuisance that impedes its confident interpretation. But we also know that iron can catalyze the production of certain toxic oxygen compounds, so its behavior under stress may be pathophysiologically relevant, as well. *Dr Jacqueline J. Smith and co-authors* from the Hayden VA Medical Center (Phoenix) and the University of Kansas Medical Center (Kansas City) responded to the observation that the amount of ferritin in alveolar cells and on the alveolar surface is increased in a variety of respiratory disorders. They studied whether *in vitro* exposure to hypoxia or nitric oxide (NO) would induce ferritin accumulation or release by human alveolar macrophages (AM) or by a (lung-cancer-derived) pulmonary epithelial cell line (A549). They measured changes in cell content of iron and ferritin, as well as ferritin content of supernatant culture medium after exposure to hypoxia (1% or 10% O_2, 18 hours) or the NO-donor, S-nitroso-N-acetylpenicillamine (SNAP, 0.01-1.0 mM, 18 hours).

Exposure to hypoxia (1% O_2) increased ferritin content in both cell types by about fourfold without changing iron content. Treatment with SNAP, the NO donor, increased ferritin content of A549 cells in a dose-dependent manner, bur *decreased* the iron and ferritin content of alveolar macrophages. Ferritin was increased in the supernatant medium from the AMs. Pretreatment of cells with *N*-acetylcysteine (500 μM) reduced hypoxia-induced ferritin accumulation in alveolar cells and completely inhibited NO-induced ferritin accumulation in A549 cells. These findings indicate that exposure to 1% O_2 can increase ferritin content of alveolar cells whereas NO can increase ferritin content (A549 cells) or decrease ferritin content (AM).

These results might at first blush seem a bit confusing. An editorial by *Professor Des R. Richardson*, of the Children's Cancer Institute Australia for Medical Research, puts them in context of a discussion of iron regulatory proteins, iron responsive elements, and oxidative defenses. The paper may be found on page 309, and Dr Richardson's editorial appears on page 289.

Fish oil and mesangial cells

Since the original description by Dyerberg and Bang that omega-3 fatty acids limit platelet activation and thus might be helpful in preventing heart disease, fish oil has been suggested for many ills, among them IgA nephropathy. In that context, a number of studies have suggested that it may actually work, but few studies shed light on why that might be.

Ahad N. K. Yusufi and colleagues from the Mayo Clinic have used an antithymocyte serum (ATS) model of mesangial proliferative glomerulonephritis to show that fish oil inhibits mesangial cell (MC) activation and proliferation, reduces proteinuria, and decreases histologic evidence of glomerular damage. Beginning on page 318, they now describe their work trying to characterize the mechanism(s) of the antiproliferative effect of fish oil—presumably worked by docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), the oil's predominant ω-3 polyunsaturated fatty acids.

DHA and EPA were provided to MC in tissue culture as albumin-fatty acid complexes. Although these two fatty acids shared some effects, there were many for which DHA was effective at a substantially lower concentration. Low-dose (10-50 μM) DHA, but not EPA, inhibited basal and EGF-stimulated mesangial cell proliferation, as assessed by [^{3}H]-thymidine incorporation. At higher doses (100 μM), EPA and DHA were equally effective in suppressing basal and EGF-stimulated MC mitogenesis. Low-dose DHA, but not EPA, decreased ERK activation by 30% ($P < .01$). JNK activity was increased by low-dose DHA, but not by EPA. p38 activity, in contrast, was not significantly altered by either DHA or EPA. Cyclin E activity was inhibited by low-dose DHA, but not EPA. DHA increased expression of the cell cycle inhibitor p21, but not p27; EPA had no effect on p21 or p27 levels.

In many systems, the effect of fish oil is to substitute EPA for arachidonic acid in cell membranes, leading to major changes in the cell's eicosanoid production. This example is quite different, in that DHA appears to be the more important actor. The authors propose from the above observations that the differential effect of low-dose DHA versus EPA in suppressing mesenchymal mitogenesis is related to downregulation of ERK and cyclin E activity, and induction of p21.

For the editors
Dale E. Hammerschmidt
Editor-in-Chief