Response:

Prevention of aspergillosis

We agree with Winston et al’s emphasis on prevention; however, we do not agree that the data available definitively demonstrate the superiority of itraconazole. The design of our trial impacted the overall outcomes. Most important was the use of antifungals during conditioning, given the trend to early toxicities in itraconazole recipients who received cyclophosphamide.1,2 This trend appeared to decrease in patients enrolled after the protocol was modified to initiate antifungals after conditioning; however, there were too few patients enrolled to enable comparisons.

We do not have the data necessary to compare toxicities between the 2 trials. Winston et al3 reported the adverse events thought possibly to be related to the study drugs, and we reported the number of patients who developed toxicities independent of cause. More itraconazole recipients developed tripling of baseline total bilirubin (143 of 151, 95%) compared with fluconazole recipients (128 of 143, 86%; P = .02). The incidence of hepatotoxicities in patients who received fluconazole is not different compared with similar patients in our center; the high proportion reflects the definition of “hepatotoxicity.”

Despite the differences in timing and dosing of antifungals in the 2 trials, the outcomes of patients who received itraconazole were remarkably similar. Both studies reported high rates (24%) of gastrointestinal (GI) side effects. Also, the invasive fungal infections (IFI) incidences in itraconazole recipients were not different (13% vs 9%). The only large difference was the incidence of IFI in fluconazole recipients (16% vs 25%, Winston et al study). Outcomes in the Winston et al study were driven by an unusually high number of candidemias (8 of 67 patients, 12%)—much greater than in our study (2.6%) or in prior trials.5,6

While we have learned important lessons, we do not think that the data generated by either trial support the conclusion that itraconazole prophylaxis is a better strategy. In the Winston et al study,3 outcomes may have been impacted by bias between the patients randomized, as more fluconazole recipients received unrelated donor transplants (likewise, increased graft-versus-host disease [GVHD] and therapies). Moreover, the 140 patients enrolled did not provide the power to demonstrate superiority. Finally, it is notable that the apparent IFI protection was not associated with a trend to improved survival. We believe that the conclusion of superiority should be supported at least by a trend toward decreased transplant-related mortality in order to negate the possibility that decreased infection rates are associated with excessive antifungal-related toxicities.

The data presented for the 73 follow-up patients are compelling. Based on the results of Winston et al’s trial, we would expect at least 4 IFIs in this cohort. This raises questions: What were the methods of surveillance and the duration of follow-up? Were there other patient or environmental factors that changed? Without having a contemporaneous control, it may be difficult to identify the causative factor(s).

We agree with the assertion that prevention is better than treatment, and we are intrigued by the trends in the Winston et al study3 and the subset of patients enrolled later in our trial. However, we believe that policy decisions should be based on data obtained from properly designed randomized trials rather than trends demonstrated in underpowered studies or retrospective cohort analyses. It is not clear to us that the risk-benefit ratio favors itraconazole for prophylaxis, but we share our colleagues’ enthusiasm that a newer azole may accomplish our common goal of preventing aspergillosis.

Kieren A. Marr and Michael Boeckh

Correspondence: Kieren Marr, Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D3-100, Seattle, WA 98109.

References


To the editor:

Phosphatidylserine externalization in cardiolipin-deficient cells

We were intrigued by the recent article by Kuijpers et al1 on the binding of annexin V to neutrophils obtained from Barth syndrome patients. The authors reported that freshly isolated neutrophils from these patients were readily labeled with annexin V (a phosphatidylserine [PS]–binding protein) in the absence of other indices of apoptosis and concluded that these cells expose an alternative ligand for annexin V that is distinct from PS. We suggest that other recent findings could be invoked to explain these novel observations in cardiolipin (CL)–deficient Barth syndrome cells.

PS externalization is an important “eat me” signal on apoptotic cells and serves to alert neighboring macrophages. Neutropenia (reported to be a common finding in Barth syndrome, albeit not a prominent feature in the cohort examined by Kuijpers et al) could thus be linked to the aberrant exposure of PS on circulating neutrophils. Conversely, the lack of PS externalization in neutrophils from chronic granulomatous disease (CGD) patients could contribute to defective clearance and the formation of granulomas in these individuals.2 However, Kuijpers et al argue that annexin V...
binding to Barth syndrome neutrophils is unlikely to reflect PS externalization because macrophages failed to engulf these cells upon cocultivation in vitro. While the existence of alternative ligands for annexin V cannot be excluded (indeed, annexin V was recently found to associate with the cytoplasmic domain of the β5 integrin),8 we wish to point out that the failure of macrophage clearance does not provide a priori rule out the presence of PS molecules on the cell surface. Previous studies in neutrophil-like HL-60 cells and other cell lines have shown that PS oxidation is an integral part of the apoptosis program; hence, exposition of PS alone in the absence of PS oxidation may not suffice to mediate clearance of cell corpses.4,5 Moreover, Kagan and his associates (Borisenko et al6) have provided strong evidence that macrophages have a sensitivity threshold for PS that facilitates reliable discrimination of apoptotic cells; it is thus conceivable that Barth syndrome neutrophils expose subthreshold amounts of PS, detectable by the annexin V assay, but insufficient to mediate macrophage clearance. Macrophage recognition may also depend on cofactors (e.g., annexin I, a caspase-dependent “eat me” signal that colocalizes with PS on apoptotic cells)7 that are not expressed on freshly isolated Barth syndrome cells. Finally, caspase-independent PS externalization in neutrophils has been demonstrated previously,2 and therefore the absence of other signs of apoptosis cannot be taken as evidence that cells fail to expose PS.

The findings of Kuipers et al1 raise the interesting possibility that mitochondrial CL deficiency is directly linked to PS externalization. In support of this notion, recent studies in yeast with a disruption in the gene encoding CL synthase and therefore lacking CL in mitochondrial membranes have revealed a labilization of cytochrome c binding in these organelles.8 Moreover, recent studies in mammalian cells show that cytosolic cytochrome c may serve as a catalyst for PS oxidation with subsequent cell surface externalization of PS.9 It would therefore be of considerable interest to assess whether (constitutive) mitochondrial extrusion of cytochrome c could facilitate the egress of PS, and consequently the binding of annexin V, in CL-deficient Barth syndrome neutrophils. In addition, further analyses of yeast that lack CL synthase, or yeast that harbor a taz1 mutant and therefore display aberrant CL metabolism,10 may also shed some light on the mechanism of PS externalization in CL-deficient and -proficient cells.

Bengt Fadeel, Maria B. Karpova, Mari Enoksson, and Sten Orrenius
Correspondence: Bengt Fadeel, Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden; e-mail: bengt.fadeel@imm.ki.se

References

Response:

Cardiolipin and annexin V: unrelated issues

The issue raised that annexin V may still recognize phosphatidylserine (PS) on the neutrophils freshly obtained from patients suffering from Barth syndrome (BTHS) is of course correct as was discussed in our paper. In particular, the point raised as to whether oxidized or nonoxidized lipids could be responsible for the basal annexin V staining has been mentioned by us as a possibility that we cannot exclude. On the other hand, oxidized PS would have been detected by the high-performance liquid chromatography–mass spectrometry (HPLC-MS) methods used. To date, we were obviously not able to detect this lipid form. We cannot answer the question of how much of the oxidized PS makes more sense. However, the recent findings using PS receptor (PSR) knock-out mice make the interpretation again puzzling. These mice showed abnormal brain development and neonatal lethality in a fraction of PSR−/− mice.3 Subsequent studies in another murine PSR knock-out strain demonstrated similar developmental defects in brain and other organs but an intact clearance reaction of apoptotic bodies by PSR−/− macrophages.4 The latter findings question the actual role of the PSR. Its role may be far less important—if it exists—in the clearance mechanisms of apoptotic material than was suggested previously by the group of Fadok and Henson (Fadok et al5) because of redundancy in these biologic processes.

We do not believe that cardiolipin (CL) deficiency is directly linked to PS externalization because the circulating lymphocytes and monocytes in BTHS—being as CL-deficient as the circulating neutrophils—did not avidly bind annexin V. If this cellular difference is not taken into account, any speculation on the mechanism related to CL becomes less valid.

The suggestion of a catalyst function of cytochrome c in the cytosol to initiate PS oxidation may well exist in certain model systems but not in BTHS neutrophils. First, if so, this would (along the same line of reasoning of Fadeel et al) result in macrophage-mediated PS recognition, which is clearly not the case in our experiments. Second, in
neutrophils there is little if any cytochrome c present, turning CL-defective cells into a ready-to-release state or making “labilization” of cytochrome c far less likely.

In sum, there is not yet a clear answer to the observations made in BTHS neutrophils. To date, the hypothesis of an alternative ligand fits best with our results.

Taco W. Kuijpers

Correspondence: Taco W. Kuijpers, Emma Children’s Hospital, Academic Medical Center, Amsterdam, the Netherlands; e-mail: t.w.kuijpers@amc.uva.nl

References


To the editor:

Immunochemotherapy is the standard of care in elderly patients with diffuse large B-cell lymphoma

Pfreundschuh et al recently presented the results of a German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) trial detailing outcomes with 2- or 3-weekly cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP] chemotherapy, with or without etoposide, in elderly patients with aggressive non-Hodgkin lymphoma (NHL).¹ The predefined analyses failed to demonstrate a significant superiority of any of the regimens in terms of the primary end point of time to treatment failure (TTF). However, unplanned multivariate analyses showed a relative risk reduction for event-free and overall survival after CHOP-14 (biweekly) compared with CHOP-21 (every 3 weeks). Another study by the Japanese Clinical Oncology Group (JCOG) was terminated early by the Data and Safety Monitoring Committee (DSMC)² because the first interim analysis revealed shorter progression-free survival with CHOP-14 compared with CHOP-21 (24 vs 34 months, respectively). Furthermore, CHOP-14 was only marginally superior to CHOP-21 in a group of younger patients with good-risk aggressive lymphoma in another DSHNHL study.³ Thus, it seems premature to conclude that CHOP-14 should be considered the new standard for elderly patients with diffuse large B-cell lymphoma (DLBCL), and other regimens may challenge this title.

The DSHNHL trial randomized 831 patients, but efficacy and safety analyses were performed on only 689 patients with confirmed histology of aggressive lymphoma.¹ The majority of the patients had low-risk disease defined as an age-adjusted international prognostic index (aaIPI) of 1 or less, and fewer patients had adverse characteristics than in our study comparing CHOP plus rituximab (R-CHOP) with CHOP alone in patients ages 60 to 80 years.⁴⁵ Of our patients, 60% had high-risk disease (aaIPI ≥ 2; Table 1). Patients were analyzed as intent-to-treat (all patients) compared with trial-defined population in the DSHNHL study. After a median follow-up of 4 years, event-free and overall survivals are significantly longer for R-CHOP patients (P = .00008 and P = .008, respectively).⁶ Survival benefit with R-CHOP was found independent of age or comorbidity scores.⁷ Importantly, these improved outcomes were achieved with minimal additional toxicity.²⁶ The benefit of adding rituximab to CHOP has been confirmed by several large randomized trials. A US Intergroup study randomized DLBCL patients aged 60 years or older to receive either 6 to 8 cycles of CHOP or R-CHOP chemotherapy.⁸ Responding patients were randomized to receive either rituximab maintenance or no further treatment. If this study was not designed to directly compare R-CHOP with CHOP, additional analyses reveal that R-CHOP induction significantly prolongs TTF and overall survival.⁹ A large historical population-based analysis was conducted in British Columbia comparing CHOP or R-CHOP in DLBCL patients and confirmed the Groupe d’Etude des Lymphomes de l’Adulte (GELA) results.⁵ The MabThera (rituximab) International Trial (MiNT) determined whether the benefits seen with R-CHOP in elderly patients could be extended to younger patients with DLBCL. A preplanned interim analysis has demonstrated a statistically significant improvement in TTF for patients receiving rituximab.¹⁰ Accordingly, further randomization has been stopped by the DSMC.

These results indicate that the addition of rituximab to CHOP is associated with substantial and repeatedly demonstrated clinical benefit and that immunochemotherapy should be considered as the new standard of care in DLBCL patients.

Bertrand Coiffier and Gilles Salles, for the GELA

Correspondence: Bertrand Coiffier, Hematology Department, CH Lyon-Sud, 69495 Pierre-Benite, France; e-mail: bertrand.coiffier@chu-lyon.fr

B.C. is part of the speaker bureau of Roche and Genentech. G.S. is part of the speaker bureau of Roche. The GELA has received unrestricted grants from Roche and Genentech.

References

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