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In Vitro Hypersensitivity to Oxygen of Fanconi Anemia (FA) Cells Is Linked to Ex Vivo Evidence for Oxidative Stress in FA Homozygotes and Heterozygotes

To the Editor:

The report by Whitney et al[1] provides a brief mention of a phenotypic feature of Fanconi anemia (FA) cells related to oxygen hypersensitivity. However, one[2] of the two citations used may lead the reader to overlook the in vivo relevance of this phenomenon, as the investigators attributed a ''secondary'' role for oxygen sensitivity in FA cells. A previous report by Liu et al[3] devoted a section to the abnormalities of oxygen metabolism in FA cells; however, no clear distinction was made between the results of in vitro studies versus the ex vivo data as reported by Korkina et al.[4] Therefore, it is worthwhile to consider the subject of oxidative stress in FA with a reappraisal of the consistency between in vitro and ex vivo evidence.

An involvement of oxidative stress in FA has been long suspected, starting from the pioneering study by Nordenson,[5] who reported an improvement of chromosomal instability following addition of catalase or Cu,Zn superoxide dismutase (Cu,ZnSOD) to cell cultures. Further studies pointed to analogous results by exposing FA cells to low-molecular-weight antioxidants or to a decreased oxygen level.[6-9] Schindler and Hoehn[10] showed a G_2 cell-cycle delay in FA cells, which was counteracted by culturing cells in 5% O_2, and a recent report from the same group suggested a major role for free iron, and not for superoxide- or H_2O_2-forming systems in inducing G_2 arrest in FA cells.[11] Overall, the information available from in vitro studies suggested excess O_2 (Fe?) sensitivity of FA cells, although some studies led to controversial conclusions, eg, reporting the lack of oxygen effect following viral transfection[2] or on *FAC* gene expression.[12] However, these data may have been biased by virus-induced cell immortalization.[12]

A series of ex vivo studies provided evidence that an abnormality in O_2 metabolism is not merely a cell culture artifact. Rumyantsev et al[13] first reported that freshly drawn white blood cells (WBCs) from FA patients and from their parents produced excess reactive oxygen species (ROS) as detected by luminol-dependent chemiluminescence (LDCL). These data were confirmed on an extended set of Italian FA families,[4] mostly belonging to the FA(A) group.[14] Subsequent studies corroborated those early reports:

(1) Circulating WBCs from FA(A) patients displayed excess levels of the oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine (8OHdG), correlated with LDCL activity as well as with chromosomal instability.[15] These data were consistent with the observation of excess 8OHdG formation in FA(A) cell lines submitted to H_2O_2 stress.[16] Thus, both ex vivo and in vitro evidence pointed to a direct link between ROS formation, oxidative DNA damage, and chromosomal breakages in FA.

(2) Clastogenic factor (CF) was detected in plasma from FA(A) patients, and their siblings and parents.[17] CF was obtained by plasma ultrafiltration, increasing the frequencies of chromosomal breakages in cells from healthy donors. The loss of clastogenic activity by SOD or low-molecular-weight scavengers suggested that plasma CF in FA homozygotes and heterozygotes could be related to the occurrence of in vivo oxidative stress.

(3) Tumor necrosis factor-α (TNF-α) was found to be significantly increased in FA patients versus control plasma.[18] Because TNF-α is a recognized effector of ROS release from phagocytes, its elevated levels may be associated with phagocyte activation. Which relationship, if any, exists between TNF-α and CF is a question deserving further investigation. However, the present data suggest that FA patients have a ''prooxidant plasma,'' possibly arising from activated phagocytes.

Lastly, the observation of erythrophagocytosis in bone marrow from early stage FA patients[19] can be viewed as an in vivo condition consistent with a chronic activation of phagocytes.

One limitation in the above data is that studies have been conducted so far either on FA(A) cells[4,14-16] or plasma,[17] or on cells with unknown FA gene defects.[13,18,19] Therefore, further ex vivo studies are required to ascertain any differences in oxidative activity as related to the other FA subtypes.

Notwithstanding the above limitation, the available evidence points to oxidative stress as a major phenotypic hallmark in FA that cannot be overlooked in FA clinical history. The major role for neutrophils in ROS formation, their enhanced depletion in FA progression, as well as the multiple cellular interactions of neutrophils (with, eg, platelets, endothelium, and chondrocytes), altogether make the neutrophil a prime candidate as the affected cell in FA. Ongoing investigations on the roles for FA neutrophils are expected to provide relevant information on the pathogenesis of this disorder.

Giovanni Pagano
Italian National Cancer Institute
G. Pascale Foundation
Naples, Italy
Ludmila G. Korkina
Russian Institute of Pediatric Hematology
Moscow, Russia
Paolo Degan
IST
Genoa, Italy
Domenico Del Principe
2nd Rome University Medical School
Rome, Italy
Barbara Lindau-Shepard
Department of Veterans Affairs
Samuel S. Stratton Medical Center
Albany, NY
Adriana Zatterale
Cytogenetics Unit, Elena D'Aosta Hospital
Naples, Italy
Claudio Franceschi
Institute of General Pathology
University of Modena Medical School
Modena, Italy

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Platelet Antibody Testing in Idiopathic Thrombocytopenic Purpura

To the Editor:

Although it has been accepted for decades that most cases of idiopathic thrombocytopenic purpura (ITP) are caused by autoantibodies against platelets,[1] the clinical utility of serologic testing in ITP never has been established.[2] A recent report by Brighton et al[3] analyzed predictive characteristics of direct and indirect tests for platelet-associated IgG (PAIgG) and an antigen capture test (MAIPA) designed to detect autoantibodies specific for platelet glycoproteins IIb/IIIa or Ib/IX. It was concluded that the MAIPA was better than tests for PAIgG in discriminating immune from nonimmune thrombocytopenia.[3]

We recently analyzed the clinical utility in ITP of a commercially available serologic test for PAIgG, the Capture-P (Immucor, Inc, Norcross, GA). This is a solid-phase red blood cell adherence assay that is used to detect alloantibodies for platelet crossmatching and also has been reported to have high sensitivity and specificity in ITP.[4] In this test, microtiter wells are coated with an agent that binds platelets from autologous (direct method) or allogeneic (indirect method) platelet-rich plasma. Adherent platelets create a substrate for capture of antiplatelet IgG from test plasma. Platelet-bound IgG is detected by subsequent adherence of indicator red blood cells coated with anti-human IgG.

A search of the University of Iowa Hospitals' medical record department computer data base retrieved 359 records that contained results of Capture-P tests performed between August 1989 and July 1994. Of these, 94 records that contained ICD-9 coded primary diagnoses of primary thrombocytopenia (287.3) or thrombocytopenia, unspecified (287.5), were reviewed. Patients were retrospectively classified into three groups: (1) ITP (platelet count $<$150,000/μL, normal or increased numbers of bone marrow megakaryocytes, and no other known cause of thrombocytopenia) (n = 46); (2) thrombocytopenia of other established cause (n = 15); or (3) thrombocytopenia of undetermined cause (incomplete data or criteria for ITP not met, and no other cause of thrombocytopenia established) (n = 33). Group 3 was excluded from analysis.

The sensitivity and specificity of the Capture-P for the clinical diagnosis of ITP were 37% and 67% for the direct method, and 43%

Table 1. Predictive Characteristics of Serologic Tests for Platelet Autoantibodies

Test	Method	Sensitivity	Specificity	Likelihood Ratio*
Capture-P	Direct	17/46 (37%)	10/15 (67%)	1.1
Capture-P	Indirect	20/46 (43%)	9/15 (60%)	1.1
CELIA†	Direct	60/81 (74%)	12/46 (26%)	1.0
ELISA‡	Indirect	30/88 (34%)	41/53 (77%)	1.5
MAIPA§	Modified	33/71 (47%)	34/40 (85%)	3.1

* Likelihood ratio = sensitivity/(1 − specificity).

† Competitive enzyme-linked immunoassay for PAIgG (data from Brighton et al[3]).

‡ Enzyme-linked immunosorbant assay for PAIgG (data from Brighton et al[3]).

§ Modified monoclonal antibody immobilization of platelet antigens (data from Brighton et al[3]).