Commentary   Open Access

Neutrophil Biology: JAGN1 Deficiency is Responsible for Neutropenia

Payel Sil*

Graduate Assistant, Department of Infectious Diseases, University of Georgia, Athens, GA 30602, USA.

*Corresponding author: Payel Sil, Department of Infectious Diseases, University of Georgia, Athens, GA 30602, USA, Tel: +1 706-542-3000; E-mail: payel@uga.edu

Commentary

Granulocyte-Macrophage colony stimulating factor can salvage JAGN1 defect in bone marrow precursors causing congenital neutropenia. Neutrophils are the sentinel of host immune systems that are dispatched to surveil, engage and combat microbial pathogens. Neutropenic patients with low neutrophil counts are unable to mount neutrophil based immune responses and as a result become vulnerable to infections. JAGN1 encoding Jagunal homolog 1 expressed in hematopoietic progenitors and it's deficiency makes neutrophil incompetent effector cells. In JAGN1 mutant mice, Penniger et al. demonstrated that the defect in JAGN1 function is rescued by granulocyte/ macrophage colony stimulating factor (GM-CSF) when afflicted by *Candida albicans*. JAGN1 mutant neutrophils are characterized by fewer granules, aberrant N-glycosylation of multiple protein patterns, apoptosis and are consequently unable to battle the pathogens. The authors showed that only GM-CSF helps in maturation of Myeloperoxide (MPO) containing secondary granules and thus, rescues the impaired reactive oxygen species (ROS) production [1]. The paper presented compelling evidence the JAGN1 defect was due to alterations in the N- glycosylation causing malfunction in neutrophil homing and effector roles [1,2]. Further, the study was extended in Severe Congenital Neutropenia (SCN) patients, whose bone marrow granulocytes was challenged with Candida, and then treated with GM-CSF *in vitro*. It was, thus, established that this new-found neutrophil regulator, JAGN1, is responsible for congenital neutropenia [1,2].

Given the viability challenge posed by JAGNI knockout mice embryos, Penniger et al., established a mouse model with exon 2 deletion in JAGN1 gene and validated that the blood cell specific JAGN1 loss did not affect other hematopoietic cell lineages. These JAGN1Δhem mice when challenged with *Candida albicans* showed an inability to cope with the pathogen, as seen in neutropenia. Apart from developing the mice model, the novelty of the study lies in the ingenious approach of administering GM-CSF *in vitro* in JAGN1Δhem mice neutrophils challenged with fungi (Figure 1).

GM-CSF is an established growth factor for differentiating myeloid cell precursors and is often used as a prophylactic a long with G-CSF (Granulocyte colony stimulating factor) in neutropenia patients [2-6]. To combat pathogens neutrophils congregate by chemo taxis, phagocytosis infiltrates the inflicted site and produces many anti-microbicidal proteases [4]. All these processes are defective in JAGN1Δhem neutrophils due to their limited ROS activity. Neutrophil ROS production is driven by NADPH oxidase, and it is instrumental in various host defense mechanisms like pathogen killing, Neutrophil extracellular trap (NET) formation, inflammasome activation etc [4]. Neutrophils undergo NET formation in response to heavy ROS production and degranulation in presence of *Candida albicans* (spore or hyphae stage) [4,6]. Failure to produce ROS leads to concurrent infection as seen in chronic granulomatous disease (CGD), Chediak Higashi disease (CHD), and neutrophil specific granule deficiency

(SGD) [4]. Similarly, due to certain genetic deficits, neutrophils in SCN patients deviate from their traditional role as pathogen defenders and succumb to the JAGN1 mutation, leaving the onus of removing any or subsequent foreign pathogens to the other phagocytic cell types (such as macrophages, dendritic cells, NK cells and etc).
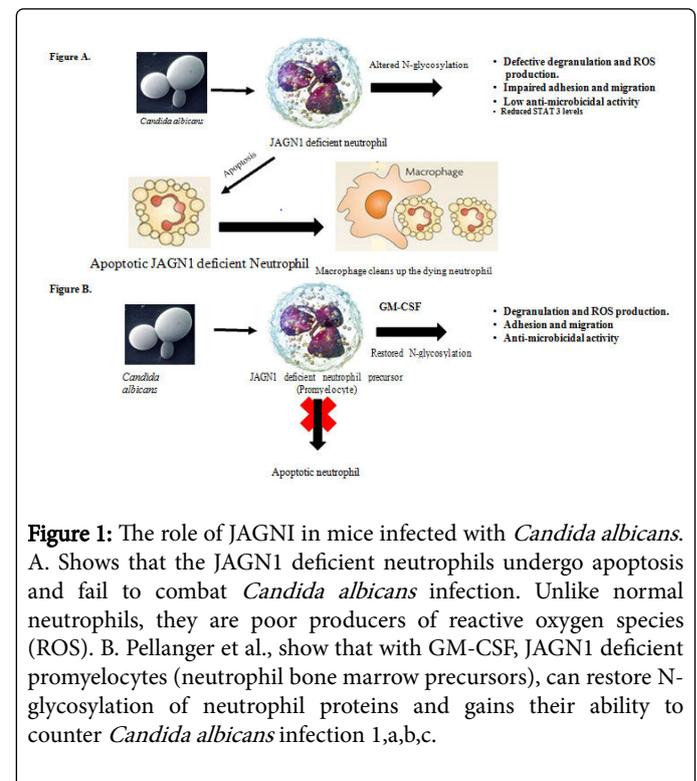

Figure 1: The role of JAGNI in mice infected with *Candida albicans*. A. Shows that the JAGN1 deficient neutrophils undergo apoptosis and fail to combat *Candida albicans* infection. Unlike normal neutrophils, they are poor producers of reactive oxygen species (ROS). B. Pellanger et al., show that with GM-CSF, JAGN1 deficient promyelocytes (neutrophil bone marrow precursors), can restore N-glycosylation of neutrophil proteins and gains their ability to counter *Candida albicans* infection 1,a,b,c.

Pellanger's team investigated the recruitment of phagocytes post-Candida infection in the JAGN1Δhem mice and found few neutrophils to be present. The recruited JAGN1Δhem neutrophils were able to produce ROS and phagocytose but were poor at killing the fungi owing to their reduced number of primary, secondary and tertiary granules.

Consistent with these findings Klein et al., published data that supported the association of the JAGN1 mutation with SCN patients [2]. They found that the promyelocytes and myelocytes undergo maturation arrest which is not due to the absence of neutrophil differentiation and maturation factors (ELANE, HAX-1 and G6PC3) [2,7]. Recent research reveals that the neutrophils undergo apoptosis due to depolarization of the mitochondrial membrane potential which supports Pellanger's study showing N-glycosylation alternation [2]. This causes up-regulation of certain Gal-α-1,3- Gal terminated N-glycoforms and reduction in biantennary sialic acid ending structures while undergoing Golgi-ER processing [1,2]. Thus, N-glycoprotein alteration results from faulty membrane trafficking due to the loss of

JAGN1. Later studies confirmed that JAGN1 interacts with a vesicular membrane trafficking complex known as Coat protein I (COPI) [2]. Both Penniger, et al. and Boztug K, et al. demonstrated that ER stress induces neutrophil apoptosis [1,2].

Global glycoproteomic profile show that impaired N-glycosylation specifically affects molecules adhesion, migration molecules (CD177, CD11b and CD18), tissue remodeling and cytotoxic effector functions [1,2,8-10]. Consequently, vital proteins such as neutrophil collagenases (Mmp8), matrix metalloproteinase 9 (Mmp9), Lactoferrin (Ltf), lipocalin2 (Lcn2), haptoglobin (Hp) and neutrophil granule protein (ngp) display maturation defects.

Abnormal degranulation can lead to ineffective presentation of the adhesion and chemotactic molecule on the neutrophil surface [4]. Neutropenic patients have sloppy neutrophil driven immune systems, as their neutrophils undergo apoptosis upon encountering opportunist pathogens.

Another intriguing finding was that JAGN1Δhem neutrophils have high expression of GM-CSF-R and upon treatment with GM-CSF, impaired MPO activity was restored. Reinstating N-glycosylation of neutrophil protein and GM-CSF supplementation renewed the anti-microbial role of the neutrophils (Figure 1) [1,2]. G-CSF R is compromised in SCN so oftentimes, G-CSF is administered therapeutically [1,7]. CGD, CHD, SGD and MPO deficient patients are commonly treated with G-CSF and GM-CSF to replenish their neutrophils [4,7]. *In vitro* studies show that the G-CSF primes neutrophils and enhances their ROS production [8]. The IL-17- IL23-G-CSF axis increases production of both G-CSF and GM-CSF, and thereby, produces a cytokine environment conducive for neutrophil proliferation [4,6]. Therapeutic strategies to modulate the IL-17- IL23-G-CSF axis may advance our understanding of the exclusive role of GM-CSF in JAGN1 mutation 4. In mice it has been shown that GM-CSF upregulates neutrophil migration markers such as CD 54 and Dectin 2, however, it's role in SCN patients requires evaluation [8].

Although some details remain unresolved, Penniger et al., have paved the way by revealing the gene responsible for SCN. Growing understanding of the molecular pathway will help researchers in identifying the key player(s) involved in neutrophil effector functions linked to their bone marrow precursors. This fascinating new finding can be channeled to understand diseases related to aberrant membrane trafficking in congenital (Hermansky Pudlak Syndrome II, Cohen syndrome etc.) or acquired neutropenia (cancer patients undergoing chemotherapy) and to develop therapeutics targeting early stages of human granulopoiesis [2,4].

References

1. Penniger JM, Wirnsberger G, Zwolanek F, Stadlmann J, Tortola L, et al. (2014) Jagunal homolog 1 is a critical regulator of neutrophil function in fungal host defense. Nat Genet 46: 1028-1032.

2. Boztug K, Järvinen PM, Salzer E, Racek T, Mönch S, et al. (2014) JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nat Genet 46: 1021-1027.

3. Castagnola E, Dufour C (2014) Role of G-CSF GM-CSF in the management of infections in preterm newborns: an update. Early Hum Dev 90 Suppl 2: S15-17.

4. Mayadas TN, Cullere X, Lowell CA (2014) The multifaceted functions of neutrophils. Annu Rev Pathol 9: 181-218.

5. Cain DW, Snowden PB, Sempowski GD, Kelsoe G (2011) Inflammation triggers emergency granulopoiesis through a density-dependent feedback mechanism. PLoS One 6: e19957.

6. Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, et al. (2014) Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol 15: 1017-1025.

7. Triot A, Järvinen PM, Arostegui JI, Murugan D, Kohistani N, et al. (2014) Inherited biallelic CSF3R mutations in severe congenital neutropenia. Blood 123: 3811-3817.

8. Yao Y , Matsushima H, Ohtola JA, Geng S, Takashima A, et al. (2014) Neutrophil priming occurs in a sequential manner and can be visualized in living animals by monitoring IL-1β promoter activation. J Immunol 194: 1211-1224.

9. He P, Srikrishna G, Freeze HH (2014) N-glycosylation deficiency reduces ICAM-1 induction and impairs inflammatory response. Glycobiology 24: 392-398.

10. Brinkmann V, Zychlinsky A (2012) Neutrophil extracellular traps: is immunity the second function of chromatin? J Cell Biol 198: 773-783.