favourably with these two large international reports: response rate to ATG+CsA at 6 months was 70% compared to 35% for patients receiving CsA alone, 22% for eltrombopag alone, and 21% for androgens alone (Figure 1). Overall response rates in patients receiving these different treatment options is outlined in Figure 1, which highlights a superior response rate with the ATG+CsA combination. Figure 1 also shows there was no difference in response for patients in this older patient population receiving horse (hATG) *versus* rabbit ATG (rATG), which is in keeping with two large recently published real-life studies.[7,8] Also, survival was comparable in patients receiving horse or rabbit ATG. The higher response rate of patients receiving ATG+CsA did not translate to a significantly improved survival compared to other regimens, as already shown in an EBMT study;[9] possibly due to second treatment or improved supportive care. Further support to long-term survival also for non-responders comes from the rabbit ATG study; it showed that the 10-year survival of AA patients, classified at 6 months as non-responders to a first course of rATG+CsA, was comparable whether patients were then allografted (64% survival) or not (60% survival).[8] Late responses, improved supportive care, and second treatments could possibly explain the outcome of non-allografted patients.

In conclusion, ATG+CsA remains the treatment of choice for patients with AA, also for those over the age of 60; it should be preferred over the administration of CsA with or without androgens or eltrombopag, with or without CsA. Whether the addition of eltrombopag to ATG-CsA first line will further improve the outcome, as recently suggested,[10] will be determined by an ongoing prospective randomized trial (RACE trial, EBMT). This study also confirms that in real-life analysis, horse or rabbit ATG produce almost identical response rates and survival, also in older AA patients.

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Milk and the "Grandmother Effect"– a new contribution to the legacy of Ray Owen

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The article by Schonewille *et al.*, in the September 2018 edition of Haematologica,[1] tests the theory originally proposed by Owen *et al.* [PNAS, 1954[2]] that a "Grandmother Effect" can protect the offspring from hemolytic disease of the fetus and newborn (HDFN) [see illustration, Figure 1]. This disease is caused by maternal antibodies formed against the baby's red blood cells (rbc). One should note here that, thanks to the research of Owen and others in the 1950s, anti-Rhesus D (RhD) antibody (aka "Rhogam") prophylaxis began to be practiced for all pregnant women who are RhD- bearing an RhD+ fetus. The absence of such therapy prior to 1954 enabled Owen and colleagues to determine that, when the grandmother had RhD, the mother who was RhD- and had thereby already been "naturally" exposed to this antigen *in utero* and as a newborn via breast-feeding, was rendered incapable (for the most part) of producing antibody to an RhD encountered in her adult life as the mother of a child sired by her RhD+ husband. Subsequent to the routine application of Rhogam (anti-RhD prophylaxis) in the 1960's, the incidence of hemolytic disease of the newborn was greatly decreased, although not completely eliminated. Briefly, Rh- mothers are given a bolus of Rhogam at 28 weeks of pregnancy. If the baby that is born is Rh+(a 50:50 chance, if her husband is RhD heterozyogous), she will receive a second dose, 72 hours after giving birth. This procedure has revolutionized obstetrics and made it possible for healthy Rh+ babies to be born from multiple pregnant Rh- women.

While a boon to Ob/Gyn medicine, this highly effective clinical practice made it nearly impossible for others to

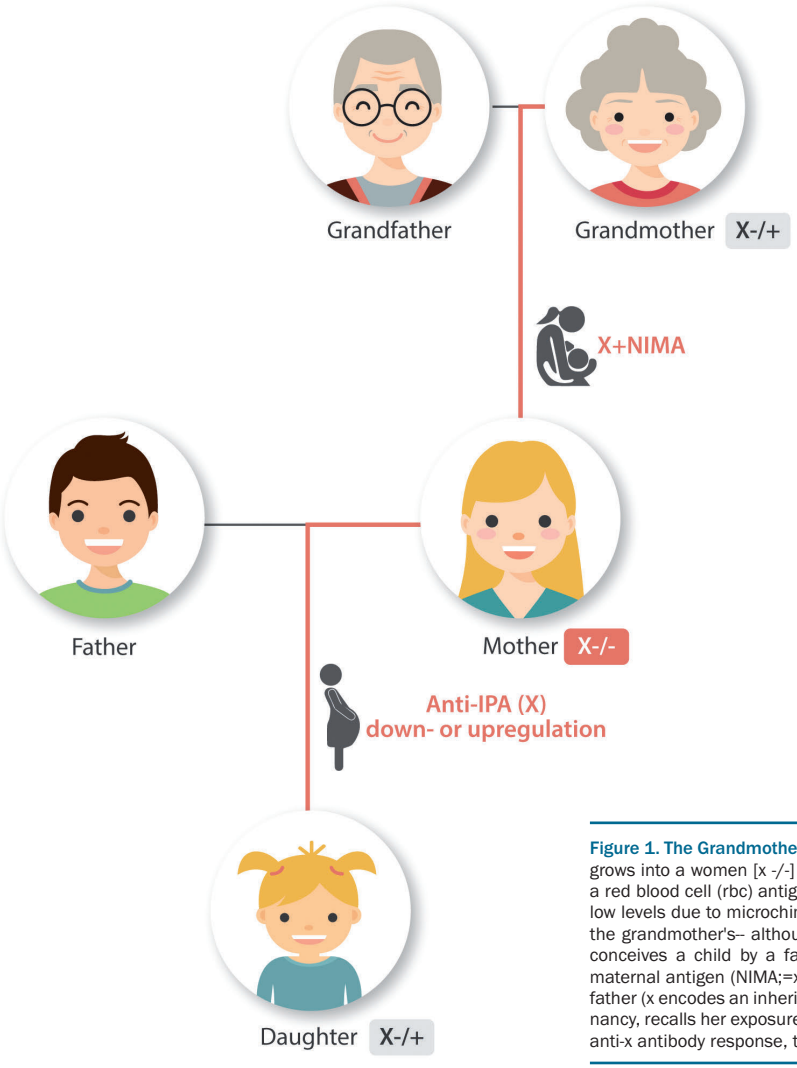

Figure 1. The Grandmother Effect. Grandmother [x -/+] gives birth to a baby girl, who grows into a women [x -/-] whose immune system bears the memory of exposure to a red blood cell (rbc) antigen (X) that she did NOT inherit but remains in her body at low levels due to microchimerism (Mc; the situation where foreign cells–in this case the grandmother's– although rare, persist in blood and tissues). Mother(x -/-) then conceives a child by a father whose rbc expresses the very same non-inherited maternal antigen (NIMA;=x). If the newly-conceived child inherits this gene from the father (x encodes an inherited paternal antigen, or IPA), then the mother, during pregnancy, recalls her exposure to this "grandmother" antigen and is less likely to form an anti-x antibody response, thus protecting the fetus.

repeat the exact test that Ray Owen had used to develop the Grandmother Effect hypothesis (even though Ray specifically invited others to do so[2]). A torrent of publications followed his, which were not able to confirm his observations of 1954. Meanwhile, other societal changes were occurring, particularly the advent of formula-fed babies, no longer exposed for long periods to breast milk.

Schonewille *et al.* therefore used a novel approach to the problem, based on the breast-feeding history of the mother provided by the grandmother. The hypothesis was that breast-feeding would condition a baby girl such that antibody response to any non-inherited erythrocyte antigens, including RhD, but also others for which prophylaxis was not done, would be inhibited. When encountered in later life, as she becomes pregnant, they hypothesized that these breast milk exposures from the grandmother would diminish her chances of developing specific antibodies to her husband's (inherited paternal) antigens (IPAs) expressed by her child's rbc.

To test whether there is indeed a cross-generational effect of the grandmother, preventing the daughter from producing antibodies to her baby's rbc and causing hemolytic disease of the newborn, Schonewille *et al.* chose to study pregnancies in cases where mothers had already developed the potential for hemolytic disease. In these women, in the period 1987-2008, their as yet unborn children were being treated by intrauterine transfusion, and their pregnancies managed with the most up-to-date techniques. The investigators studied the antibody responses of the mothers to inherited paternal antigens (IPA) expressed by their babies in relation to various factors, including the breast-feeding history of the maternal grandmother. This approach took the focus off of *in utero* exposure only and put it onto the combination of *in utero* exposure and breast-feeding. Schonewille and colleagues made two critically important and novel observations: 1) that there was indeed a Grandmother Effect limiting the mother's ability to produce anti IPA antibodies to a variety of erythrocyte antigens; and 2) that the capacity for a Grandmother Effect was time-dependent: i.e., it developed only after at least two months of breast-feeding of the mother by the grandmother. The power of the study was considerable, since the study was done on 125 3-generational Dutch families (grandmothers, mothers, and babies) exposed to 330 non-D rbc antigens and involved the measurement of antibody responses to each of these. Both highly as well as weakly immunogenic

antigens were studied so as to develop a more balanced model of what the Grandmother Effect might encompass. Out of the 549 rbc antigens not expressed by the mothers (after exclusion for antigens with no exposure from grandmother, or where antigen exposure was unknown), there were 330 known rbc antigen exposures in these women. The differences became meaningful in that, after at least two months of oral NIMA exposure via breast-feeding (i.e., per the maternal grandmother), the odds ratio of the mother (now an adult) forming an antibody to her baby's rbc was very low (0.12). Although continuation of breast-feeding of the grandmother beyond two months did not add additional protections, it was clear that breast-feeding was associated with the protections gained by the NIMA-exposed mother who had been re-exposed to the same antigen as an IPA during her pregnancy.

Discussion

The observations of Schonewille *et al.*[1] add to literature on the NIMA effect in important ways. The original observation of Ray Owen was challenged by a variety of individuals in the Ob/Gyn and blood transfusion fields. However, there was no adequate breast-feeding history of the mothers involved in Ray's study nor of the mothers whose results were claimed to have refuted Owen *et al.*[2] However, it was clear from a study by Frans Claas and colleagues in 1988, published in *Science*,[3] that in patients awaiting kidney transplant who were highly sensitized to HLA (>90% reactivity to a random panel), the existence of a NIMA among the different HLA types of cells tested by cytotoxicity assay tended to identify the rare cells to which the highly sensitized patient was unable to make an antibody. Similarly, in 1998 our lab published a paper[4] showing that the presence of a NIMA HLA haplotype on a sibling kidney allograft donor greatly increased long-term graft survival in the recipient. This was despite the fact that there was an increased rate of early transplant rejection episodes when the NIMA haplotype was present on the haplo-mismatched kidney.[4] This emphasized the split tolerance nature of the NIMA effect, where certain aspects of cellular immunity, particularly the so-called direct pathway of cellular immunity, were increased by re-exposure to the NIMA, whereas the indirect pathway, which controls antibody responses in long-term kidney allograft recipients, was impaired. Some 10 years later, Jeff Mold, Mike McCune and colleagues published a couple of papers, one in 2008[5] and one in 2010,[6] outlining in human studies strong support for the NIMA effect *in utero*, based on the development of NIMA-specific fetal T reg populations, and developing a new category for the "normal" CD4 T cell during *in utero* life—i.e., as one that has "T reg-like" qualities. Finally, Kinder *et al.*,[7] working in Sing Sing Way's lab, published a mouse study in 2015 showing exactly how such cross-generational tolerance occurs, and identifying a mechanism whereby NIMA exposure from the grandmother protects the daughter's later pregnancy from fetal loss due to a *Listeria* infection.

The elegant study by Schonewille and colleagues raises certain questions for the new era of "surrogate" motherhood. First, the surrogate mother is in a completely separate generational lineage, having been exposed by her own mother to certain non-inherited antigens that may or may not match with the antigens of the fetus which she carries. One wonders about the records of successful term pregnancies *vs*. premature delivery or fetal loss in these "two HLA haplotype mismatched" (fully allogeneic) intrauterine transplants. A second question arising from the study by Schonewille *et al.*[1] would be, "Does the surrogate mom breast-feed the baby or not, and if indeed she is breastfed by the surrogate mom, is a baby girl born to the surrogate mom now going to have outcomes of pregnancy that are related to the surrogate mom exposure?" This question can only be answered by further research on these individuals as they become adults. Another question regarding the implications of "milk-kinship" in certain Islamic and Native American societies (en.wikipedia.org/wiki/Milk_kinship), wherein a child is nursed by a woman who is not his/her biologic mother, might now be re-examined in light of the tolerogenic effects of breast-feeding described by Schonewille *et al.*,[1] particularly in those cases where the period of breast-feeding by the "wet nurse" is ≥2 months duration.

Summary

Overall, the paper by Schonewille *et al.*[1] is an important contribution to the literature on the NIMA effect. It is fitting that one of the co-authors of this paper is Jon van Rood, who passed away in 2017. Jon would have been very happy to see this paper published. He was an early champion of the NIMA hypothesis, who shared in the discovery of HLA as a key element in human transplantation, even though the Nobel prize for this was awarded to Jean Dausset. Whether or not the paper promotes a recommendation from Ob/Gyn specialists for two or more months of breast-feeding, it certainly supports such a strategy for female babies, in order to insure long-term protection of babies in the next generation from HDFN.

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