Th2 alloimmunity counteracts Th17-type response in the neonatal establishment of lymphoid chimerism

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The neonatal period of life is described as particularly susceptible to develop tolerance. This status of neonatal tolerance has been studied for decades since the discovery that semiallogeneic spleen cells inoculated at birth can induce donor-type graft acceptance. The host neonatal T-cell compartment that may account for this propensity to develop tolerance is mostly characterized by a Th2-type polarized response and a default in the cytotoxic T lymphocyte (CTL) functions that allow the establishment of lymphoid chimerism and promote donor-type graft survival. We highlighted a new role of alloreactive Th17 cells as a critical barrier to neonatal tolerance that prevents this lymphoid chimerism and further demonstrated that the Th2 immune deviation is essential to control this Th17-type response. We discuss here the potential impact of breaking the tolerizing effects of exposure of the developing offspring to alloantigens in the induction of Th17-type immunity.

Key words: Th2, Th17, chimerism, neonates, tolerance, alloimmunity

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how neonatal immunity is regulated to promote graft acceptance. Among the several factors favoring the establishment of tolerance, the persistence of circulating donor cells injected at birth is contributive. Indeed, BALB/c mice that were neonatally injected with (A/J x BALB/c) F₁ spleen cells and that retained a donor-type graft all displayed a lymphoid chimerism mostly composed of donor-type B cells that get activated upon allorecognition.² Later on, another model mimicking neonatal development demonstrated that exposure to inoculum containing mature donor T cells could induce skin graft tolerance, while microchimerism lacking T cells did not,³ suggesting that a multilineage chimerism favors long-term tolerance.

Considering the host T-cell compartment, the mechanisms underlying neonatal tolerance was largely documented to be associated with a Th2-type immune deviation and a defective CTL function,^{2,4,5} but also with the development of regulatory T cells (Tregs).^{6,7} Our group first described that blocking of IL-4, the key cytokine for Th2 cells development, totally prevented the induction of neonatal transplantation tolerance in mice inoculated at birth with F₁ spleen cells.⁸ Although several pathways involving restoration of anti-donor Th1 or CTL responses have been proposed in this breaking of neonatal transplantation tolerance, their respective roles needed to be clarified. Since IL-4 was more recently identified to inhibit the development of Th17-type responses,^{9,10} we asked whether the potential development of a Th17-type response upon IL-4 blocking could affect the neonatal establishment of tolerance

The neonatal period is considered as an essential time window for establishment of immune tolerance. A long-term tolerance to major histocompatibility complex (MHC) disparities was first described in the early fifties by Medawar, Brent and Billingham who demonstrated that neonatal injection of lymphoid cells from a MHC-mismatched donor could establish acquired skin graft tolerance.¹ Since these pioneer works, the model of transplantation tolerance induced by neonatal injection of semiallogeneic spleen cells has been studied extensively to understand

to semiallogeneic donor cells. We showed that neutralization of IL-4 at birth allowed the differentiation of antidonor Th17 cells in BALB/c neonates immunized with (A/J x BALB/c) F₁ spleen cells, and that those Th17 cells were involved in the rejection of donor-type cells normally persisting in lymphoid tissues. The neonatal Th2 bias thus opposes the development of allospecific effector Th17-type responses that control the chimerism.¹¹

It was discovered that during pregnancy and weaning, the developing immune system actually encounters alloantigens. Maternal allogeneic cells expressing non-inherited maternal antigens (NIMA) can be transferred through the placenta to the fetus and in breast milk to the neonate, leading to the existence of microchimerism. This maternal microchimerism was linked to the induction of tolerance, in particular by favoring the generation of TGF β -producing Tregs capable of bystander suppression.¹² However, exposure to NIMA does not always lead to tolerance, but rather to priming of T-cell responses. This phenomenon is thought to be dependent on the dose of NIMA encountered by the fetus and the neonate, high doses of NIMA being associated with the induction of tolerance.¹³ Conversely, newborn mice immunized with low doses of fully allogeneic cells, mimicking NIMA-expressing cells, were reported to develop cytotoxic and IFN γ -producing Th1-type responses to the alloantigens,¹⁴ both responses being possibly involved in the rejection of the allogeneic cells. As in this murine model of NIMA exposure, efficient Th1-type and cytotoxic responses can also be induced in the semiallogeneic model of neonatal tolerance we used. This could be achieved by injecting IFN γ itself¹⁵ or by CD40 engagement,¹⁶ but those treatments did not delete the donor cells. Actually, we demonstrated that the donor cells engraftment in neonates was controlled by an allospecific Th17-type response, when the Th2-type polarization is inhibited. IL-17A and neutrophils, both hallmarks of Th17 immunity, were shown as a direct effector mechanism.¹¹ The result of this Th17-type alloimmune response was a decreased lymphoid chimerism, with few donor cells still present in lymphoid tissues. This remaining chimerism that

is mostly composed of donor-type B cells and T cells can be considered as a low-dose exposure to NIMA that would potentially prime recipient effector CD4⁺ and CD8⁺ T cells. Although we did not directly examine the status of an allospecific cytotoxic response, Th17 cells were shown to induce potent cytotoxic CD8⁺ T-cell responses against tumor,¹⁷ suggesting another Th17-induced mechanism allowing control of donor cells. Furthermore, another way to promote the neonatal Th17-type response here would be the potential role of either donor-type or recipient-type Tregs, that would still be effective in IL-4-deprived mice inoculated with F₁ spleen cells and would directly favor the differentiation of alloreactive Th17 cells, as already proposed in transplantation setting.¹⁸

With our demonstration of the induction of Th17 immunity in neonates exposed to alloantigens and the strong link between Th17 cells and autoimmunity,¹⁹ speculating about the relationship between the exposure to NIMA and the development of autoimmunity later in life is tempting. Priming of fetal and neonatal T cells encountering low doses of NIMA (i.e., premature neonates and/or neonates who are not breastfed^{14,20}), as well as activation of maternal cells themselves by unshared alloantigens, could hamper the development of neonatal Tregs and Th2 cells,²⁰ and favor the differentiation of neonatal Th17 cells.^{11,13} Those Th17 cells would control the maternal microchimerism, decreasing again the dose of NIMA, reinforcing the T-cell priming and favoring the generation of autoreactive T cells, particularly Th17 cells, that would persist, and could induce autoimmune diseases. Further work on this hypothesis could provide an interesting target for understanding the deregulation at the basis of autoimmunity.

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