When Three Negatives Made a Positive Influence in Defining Four Early Steps in T Cell Development

Juan Carlos Zúñiga-Pflücker

*J Immunol* 2012; 189:4201-4202; 
doi: 10.4049/jimmunol.1202553

http://www.jimmunol.org/content/189/9/4201

---

**Why The JI?**

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

---

**References**

This article cites 37 articles, 11 of which you can access for free at:

http://www.jimmunol.org/content/189/9/4201.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by

The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Copyright © 2012 by The American Association of Immunologists, Inc. All rights reserved.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.
When Three Negatives Made a Positive Influence in Defining Four Early Steps in T Cell Development

Juan Carlos Zúñiga-Pflücker

One little, two little, three little DN cells, four little, . . . and so on, a variation on an outdated nursery rhyme, may be a good way to remind us that what now may seem like child’s play (i.e., the ability to perform prospective identification and functional characterization of early thymocytes) still resonates to this day. Going back to the early 1990s, Zlotnik and colleagues (1) published a defining characterization of immature thymocytes in The Journal of Immunology that gave us a common set of markers to effectively demarcate critical stages of early T cell development. Although initially not defined as such, these early subsets encompassed four stages that are now commonly referred to as DN1–DN4, in which DN refers to cells lacking CD4 and CD8 expression and, thus, are double negative (DN).

Following the pioneering work of Fowlkes et al. (a previous Pillars of Immunology article; Ref. 2), it became clear that T cells were derived from thymus-seeding cells that expressed neither of the cardinal markers of mature T cells, so these progenitor thymocytes became known as CD4−CD8−, DN, cells. Of note, Godfrey et al. (1) defined early DN thymocytes as also being CD3− or, as they called them, triple-negative cells. Over the years, it became a common assumption that whenever early thymocytes were being characterized further, these cells would, by definition, not express detectable levels of CD3; by extension or association, they also would not express a TCR on the cell surface (3). This made it redundant to define these cells as triple negative, when DN sufficed as long as it was clear that these were immature and, thus, not TCR-bearing cells.

The work reported by Zlotnik and colleagues (1) was built on work performed by them (4–7) and several other groups (8–17); noteworthy among these was seminal work from the Shortman laboratory (18–26). In several groundbreaking publications, in particular the work by Pease et al. (20), they began the initial dissection of the apparently monolithic DNs into additional subsets. Making use of newly characterized Abs against T cell activation markers, they began deconstructing DN thymocytes into three subsets. However, having the ability to use several Abs to define multiple subsets becomes useful only upon organizing the emerging definitions into a coherent storyline. Indeed, it is precisely this point (i.e., the definition of an easy-to-follow developmental pathway) that makes this work from Zlotnik and colleagues (1) a Pillar of Immunology.

In essence, this paper by Godfrey et al. (1) made use of markers known to be expressed on DN cells, CD44 and CD25, which revealed four easily discernable subsets (CD44+CD25−DN1; CD44−CD25+DN2; CD44−CD25−DN3; and CD44+CD25−DN4), but they added a set of significant and insightful experiments that further phenotypically, molecularly, and functionally assembled these four subsets into a developmental pathway that lent itself to a clear storyline.

The DN subsets were further characterized phenotypically by looking at the expression of CD24 (HSA), CD90 (Thy-1), and CD117 (cKit), which led the investigators to conclude that, although a subset of DN1 cells appeared to contain immature CD90lowCD117+ progenitor cells, this subset was indeed very heterogeneous. However, DN2 and DN3 cells were distinguishable by the loss of CD117 and change in cell size, from large DN2 to smaller DN3 cells. What made their work significant was their precursor-product analysis, in which DN subsets were transferred into lymphocyte-depleted (27) fetal thymus organ cultures (Ref. 28; a previous Pillars of Immunology article), which elegantly established their developmental sequence (DN1–4), their proliferative capacity (DN1 > DN2 > DN3 > DN4), and their capacity to give rise to αβ T cells (DN1–3; whereas DN4 only gave rise to γδ T cells). This last insight was further explored by performing Southern blots to examine the rearrangement status of the TCRβ in different DN subsets, which showed that TCRβ rearrangements were readily detectable at the DN3 stage.

Going beyond the end of the nursery rhyme, four little DN cells have grown into more than 10 little DN cells; at last count there are now about a dozen clearly defined DN subsets. By further analyzing for CD24 and CD117 expression, along with cell size and CD90 expression, as presciently done by Godfrey et al. (1), the DN1 subset can be subdivided into five functionally distinct subsets (DN1a–e) (29), including the canonical early T progenitor (DN1a) (30, 31). Of note, Godfrey et al. (1) alluded to the fact that the DN1 subset, although containing the earliest precursor, was by far the most heterogeneous collection of DNs. Again, if we follow along with their initial characterization, the DN2 subset appears to be separable based on CD90 and CD117 expression levels, which, not surprisingly, were used in later years used to functionally subdivide these cells into DN2a and DN2b stages (32),
corresponding to when progenitors go from being T lineage-speciﬁed to T lineage-committed cells.

The foreshadowing offered by Godfrey et al. (1) continued with their analysis of DN3 cells. They made the assertion that because this stage contained large and small cells, which also correlated to the detection of TCRβ rearrangements, and was the last stage to readily give rise to both αβ and γδ lineages in fetal thymus organ cultures, all of these events could be functionally linked. We now know this to be the case. The concept of TCRβ selection was just emerging (Ref. 33; a previous Pillars of Immunology article), and this characterization provided a clear framework with which to delineate the events occurring pre- and post-β selection, which remains in use to this day. Nevertheless, DN3 cells can be further carved into more subsets, DN3a–c, based on the expression of several markers, among these CD27 (34) and CD28 (35), along with cell size or metabolic state (36, 37). The last stage, the DN4 subset, which was known to contain precursors that rapidly gave rise to CD4− and CD8− expressing, or double-positive, cells (23), could then best be referred to as pre-double-positive cells (31); although they were surface negative for CD4 and CD8, these cells already expressed transcripts for both coreceptors.

Taken together, the initial paradigm of four DN cells (1) has led us to ~12 DN cells, which likely will continue to increase as better assays become available to measure T lineage or other outcomes and/or deﬁne novel intermediates along the way. It is precisely this ongoing quest to better deﬁne and understand these early stages of T cell development that keeps this initial work by Zlotnik and colleagues (1) relevant into the present, and it is very likely to remain equally important in the future. With this in mind, we will need to sing a different tune as we try to keep up with the more and more little DN cells that inhabit and journey through the thymus on their way to becoming mature T cells.