Signal inhibition by the dual-specific phosphatase 4 impairs T cell-dependent B-cell responses with age

Mingcan Yu,b, Guangjin Li,a,b, Won-Woo Lee,c, Ming Yuan,d, Dapeng Cui,a, Cornelia M. Weyand,b, and Jörg J. Goronzy,a,b,1

*Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305; 1Department of Medicine, Palo Alto Department of Veterans Affairs Health Care System, Palo Alto, CA 94304; 2Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul 110-799, South Korea; 3School of Industrial and Systems Engineering, Georgia Institute of Technology, Atlanta, GA 30332; and 1Lowance Center for Human Immunology, Emory University, Atlanta, GA 30322

AUTHOR SUMMARY

The immune system must undergo constant self-renewal, a process made more difficult with age. In particular, the source for T-cell regeneration, the thymus, already begins shrinking in early adulthood (1, 2). In adults and especially the elderly, therefore, new T cells are mostly generated by proliferation of the existing pool. In parallel, T cells are under continuous stress through encountering new infections or controlling persistent and latent infections (3). Because of these two processes, the biology of the T-cell changes over a lifetime. Most of these changes represent a decline in the ability to fight infections. Vaccinations hold the promise of reducing the resultant increased infectious susceptibility among the elderly, but improving vaccine responses has proven to be a challenge. Here, we investigated whether T-cell defects can be defined. We identified a signaling molecule that could serve as a target in improving vaccine effectiveness in the elderly.

A number of different strategies are being explored to compensate for immune defects in the elderly. Sustained vaccine delivery and activation of antigen-presenting cells can be improved by new adjuvants to optimize the induction of a T-cell response (4). However, T-cell proliferation and differentiation are also influenced by endogenous signaling as well as continuous and exogenous stimuli. Age-related defects in T-cell clonal expansion and differentiation, therefore, cannot be easily overcome by improved antigen stimulation alone.

We have hypothesized that a two-pronged approach is necessary to improve vaccine responses in the elderly and that, in addition to improving the delivery of the antigens recognized by immune cells, T-cell defects need to be identified and directly targeted. The objective of the current study was to identify gene products that are involved in T-cell differentiation and differentially expressed in the elderly compared with young adults. We focused on molecules that could be pharmacologically targeted. We found that the activation-induced expression of one enzyme, the dual-specific phosphatase 4 (DUSP4), was increased and more sustained in elderly CD4 memory T cells. Memory cells develop after first infection (or after vaccination), and they are then available to improve subsequent responses. They are, therefore, the major immune defense in the elderly who have experienced most infections over lifetime. DUSP4 is a nuclear enzyme that inactivates the cellular signaling molecules ERK and JNK. Its role in T-cell activation and differentiation has so far not been explored. However, we do know that sustained ERK and JNK activities help control the development of effector T cells (e.g., those cells that can regulate or stimulate other immune cells such as B cells or monocytes).

To identify the effector functions that depend on this signaling and fail to develop with increased DUSP4 activity, CD4 T cells from young adults were transfected with DUSP4 and activated. This DUSP4 overexpression reduced the ability to express immune cell stimulatory molecules CD40L and ICOS or produce IL-4, a B cell-stimulatory molecule. However, the expression of other activation markers, such as CD25, and the production of IFN-γ, a molecule maintained. These in vitro observations were confirmed in vivo in vaccinated young and elderly individuals.

All of the affected effector molecules are important for T cell-dependent B-cell responses. We have, therefore, hypothesized that increased DUSP4 expression prevents the differentiation of T cells into a type of helper cell that regulates the induction of antibody production by B cells. We found evidence for this hypothesis by studying CD4 KO mice. These mice lacked T cells with the CD4 molecule that helps them interact with other immune cells. The mice were then reconstituted with replacement CD4 T cells engineered to produce a T-cell receptor specific for a certain peptide. Before transferring the engineered cells into the mice, the cells were transduced with a DUSP4-expressing vector. Reconstituted animals were immunized and analyzed for the induction of a B-cell response. Results convincingly showed that DUSP4 expression in CD4 T cells reduces the expansion of antigen-specific B cells and production of antigen-specific antibodies.

We next investigated whether this mechanism is relevant for defective antibody responses associated with age. B cells from young adults were cocultured with activated CD4 memory cells from either young or elderly healthy individuals. We found that...
the ability to help B-cell differentiation was severely impaired in CD4 memory T cells from elderly individuals compared with young adults. Silencing the expression of DUSP4 in the T-cell population completely compensated for this defect.

In summary, our studies have identified an important negative feedback loop that limits sustained nuclear ERK and JNK signaling after T-cell activation. It does so by the activation of the gene for DUSP4 (Fig. P1). Sustained nuclear ERK and/or JNK activity is required for effective T- and B-helper activity but not for some other T-helper cell functions. Induction of DUSP4 occurs in memory CD4 T-cell responses of young adults only to an extent that permits developing T-cell activities in support of B-cell differentiation. Increased activation of the gene for DUSP4 in memory CD4 T cells in the elderly is the major defect that impairs T cell-dependent B-cell responses with age.

Our data support the notion that a two-pronged approach in vaccination—targeting T-cell activation and differentiation directly in addition to enhancing adjuvant-induced activation of antigen-presenting cells—is possible and promising as a method of overcoming incomplete vaccine responses in the elderly. DUSP4 is an identified signaling molecule that is responsible for defective T-dependent B-cell responses in the elderly. It, therefore, holds promise as a therapeutic target.