Specificity of SN50 for NF-κB?

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In the January issue of *Nature Immunology,* Ray and coworkers report data that shows a diminution in *Gata3* expression and IL-4 production by T cells after activation of p50-deficient T cells *in vitro*[1]. They also found that challenge of p50-deficient mice, in a manner that causes allergic inflammation in wild-type animals, led to an even greater decrease in T_H2 cytokines in the bronchoalveolar spaces[1]. These data provide important evidence that supports previous indications of a role for p50 (also called NF-κB1) in helper T cell effector functions[2,3].

However, the use of an inhibitory peptide (SN50) derived from the p50 protein was presented as evidence that NF-κB is the intermediate link between the TCR and selective regulation of T_H2 development (see Fig. 3)[1]. Although it was originally reported that this cell-permeable peptide inhibits nuclear induction of the NF-κB proteins, follow-up work showed that the SN50 peptide is not necessarily specific for p50 or for the NF-κB transcription factors in general[4,5]. Rather, because it competes for proteins generally involved in nuclear import, SN50 also blocks the nuclear induction of the transcription factors STAT, AP-1 and NFAT[4]. Although the peptide may be more specific at lower concentrations, 75 μg/ml of SN50 inhibited binding of nuclear extracts to AP-1 and NFAT probes, as well as the κB probe in a study with primary T cells[5]. At 37.5 μg/ml, increased AP-1 and decreased NFAT were observed[5]." Induction of AP-1 and NFAT is also linked to the TCR. STAT6, on the other hand, regulates GATA3, which, along with NFATc, plays a key role in T_H2 development[6,7]. In this context, it should also be noted that the diminution in nuclear GATA-3 that Ray and coworkers observed (Fig. 3c)[1] might be due to a generalized effect of 100 μg/ml of SN50 on nuclear-import processes[4,5].

The partial inhibition obtained at the effective concentration of 100 μg/ml of SN50 (Fig. 3)[1] suggests that comparison of the results to those obtained with other approaches to the inhibition of NF-κB (Rel) family dimers would be valid. When nuclear induction of multiple NF-κB subunits in T cells was inhibited with the use of a transgenic approach, the result was a T cell–intrinsic inhibition of IFN-γ production after antigen restimulation[8]. With immunization techniques similar to those used by Ray and coworkers[1], a modest increase in antigen-specific IgE (whose production *in vivo* depends on T_H2 responses) was observed, and no decrease in antigen-specific production of the T_H2 cytokine IL-4 was detected[8]. Of course, the use of a *trans*-dominant inhibitor cannot be equated with the targeted inactivation of a single NF-κB subunit. However, other articles on the role of NF-κB proteins in effector T cell function document a defect in IFN-γ production by T cells, and even an increase in IL-4 production[9,10]. These reports include findings on RelB and the p50-related factor p52 (NF-κB2). If SN50 inhibited only NF-κB dimers, the basis for the difference in these results is not clear. In regard to the role of RelA, although IgE production is highly dependent on T_H2 cytokines, IgE expression in RAG-deficient mice reconstituted with RelA-deficient lymphoid cells was the same as in wild-type controls[11]. In considering the activity of p50 revealed in the T_H2 component of immune responses[1–3], it is worth noting that signaling by regulatory coreceptors such as IL-1R and T1/ST2[12,13] may play a crucial role, which perhaps parallels a role played by IL-18–induced NF-κB in T_H1 responses. Overall, three points are suggested in addition to the issue of SN50 specificity. First, although particular subunits of NF-κB may play specific roles when knocked-out, collectively NF-κB in T cells may regulate both T_H1 and T_H2 responses. Therefore, the phrase "NF-κB deficiency does not affect IFN-γ production"[1] may not fully reflect the roles of NF-κB. Second, these studies indicate that individual deficiencies may reveal subunit-specific roles in effector T cells, but it is unclear whether the TCR selectively regulates just one subunit or a limited set of dimers (for example, p50-p50 and p50-RelA, but not p52-containing dimers). Third, the complex interrelationships among these subunits make it difficult to extrapolate from the results obtained with p50-deficient mice[1] to a general role for TCR-induced NF-κB. As noted by the authors, p50 can influence cells as an inhibitory p50 dimer[1]. Accordingly, it is possible that the basis for the findings of Ray and coworkers[1] reflects either a removal of repression by TCR-independent p50 homodimers already present in the nucleus of resting T cells or a stoichiometric change to p52-dominated heterodimers, rather than a role for TCR-induced heterodimers containing p50. Further studies and highly specific inhibition of NF-κB dimers will help to clarify such issues *in vitro* and *in vivo*.

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Response

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We used two independent complementary approaches in our recent *Nature Immunology* paper to show that the p50 subunit of NF-κB is critical for T_H2 cytokine production[1]. In one, we used $p50^{-/-}$ mice to investigate the ability of p50-deficient T cells to differentiate into T_H2 cells. In the second, we inhibited NF-κB translocation in wild-type $CD4^+$ T cells induced to differentiate into T_H2 cells. In both approaches, a deficiency of NF-κB limited T_H2 responses. Previously, in an *in vivo* model of allergic inflammation, we have shown that the absence of p50 abrogates eosinophilic inflammation[2], which is a T_H2 cytokine–dependent process. Both of these studies were done with mice lacking the p50 subunit of NF-κB: this eliminates the p50-p65 heterodimer—the classic NF-κB complex, which is a potent transactivator—and the p50 homodimer, known to be a potent repressor, which most likely accounts for the exaggerated IL-2 production in $p50^{-/-}$ mice[2]. We have not investigated the role of other Rel family members and, thus, comparison of our studies with studies in which the activation of multiple Rel members may be inhibited[3] is not valid.

The peptide SN50 was originally shown to inhibit nuclear import of NF-κB, NFAT and AP-1 at a high dose of 210 μg/ml (75 μM) in Jurkat cells[4]. In other studies, however, lower doses of SN50 selectively inhibited NF-κB