

whether exposure to certain mycobacteria, by inoculation, represses human atopic responses may further clarify the issue.

Shearer *et al.* raise important issues relevant to the potential and impact of immunization in childhood. Many of the tuberculin responses we observed, and their putative repression of the T_H2 -mediated atopic diseases, were likely a result of natural exposure to tuberculosis rather than of immunization. Nevertheless, we share the view that study of the role and potential of childhood vaccines in deviating T_H1 and T_H2 immune profiles in an antigen-independent manner is of real interest and may be important in future attempts to prevent and restrain both atopic and autoimmune disorders in man.

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Animal Alternatives in Germany

In his News & Comment article "Hunting for animal alternatives" (11 Oct., p. 168), Wade Roush reports on the low amount of

funding of research on animal alternatives by the governments of the United States, the United Kingdom, and the Netherlands. He also reports that attempts to replace the Draize rabbit eye test have been unsuccessful to date. As head of the National German Center for the Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET), I can comment on these two topics from the German point of view.

For the past 15 years, the German government has funded research to develop alternatives to the use of experimental animals at a rate of \$3 to \$6 million per year. ZEBET, established in 1989 as part of the German Federal Health Office, has set up an alternatives databank and operates at the national and international level with an annual budget of \$400,000. Because reduction of the numbers and the suffering of experimental animals is a key political issue in Germany, the federal government has banned safety testing of cosmetic formulations since 1987.

Roush reports that the worldwide validation trial of nine in vitro alternatives to the Draize eye tests in 36 laboratories was unsuccessful (1). In Germany, we have since conducted a trial of two in vitro alternatives to the Draize eye test, the HET-CAM test and a cytotoxicity test, to replace

the test for severely eye-irritating chemicals. The results of the trial (in 13 laboratories and on 200 chemicals) were successful and have recently been published (2). We have developed a sequential in vitro testing approach for classifying severely eye-irritating chemicals according to European Union regulations. We therefore have good reason to conclude that, depending on the toxicological problem to be evaluated, several in vitro assays are appropriately established to assess ocular irritancy for a given group of chemicals or for a specifically defined purpose, for example, to distinguish between severe and mildly irritating properties or between nonirritating and mildly irritating materials (3).

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References

1. M. Balls, P. A. Botham, L. H. Bruner, H. Spielmann, *Toxicol. in Vitro* **9**, 871 (1995).
2. H. Spielmann *et al. Altern. Lab. Anim.* **24**, 741 (1996).
3. H. Spielmann, in *In vitro Methods for Pharmacotoxicological Research*, J. V. Castell and M. J. Gomez-Lechon, Eds. (Academic Press, London, 1997), pp. 265-287.

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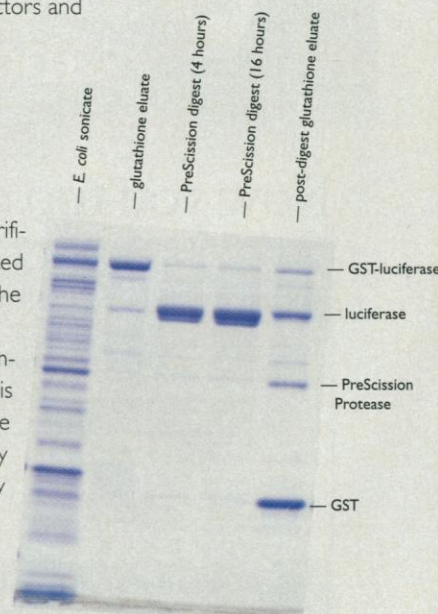
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