in the PBO + MXC hosts, the counts were comparable to those in PBO- and MXC-only mice. Similarly, in PARA + PBO mice, all sets of counts were comparable to those in PARA and PBO mice. These latter sets of results indicated that the toxicities of these two chemical combinations, at least in regard to impacts on T-cell populations *in situ*, were similar to those of any of their single chemical constituents. It is interesting to note that, with the MXC + PARA regimen, the changes in cell counts corresponded with the observed changes in SRBC-specific IgM responses. On the other hand, in the PBO + MXC group, although SRBC-specific IgM responses were decreased compared to the control, PBO, and MXC mice, there were no similar correspondence with the T-cell measures. This might suggest that the combined action of the PBO and MXC may have been directed more against the B-cell aspects of humoral responses than against T-cells; however, this still remains to be verified in more detailed studies.

In an immune response, local activated B-cells act as antigen-presenting cells for helper or cytotoxic T-cells (Goutet et al., 2005), proliferate, and differentiate into plasma cells to secrete antigen-specific antibodies. Some B-cells are activated at the T/B -cell border and migrate to form germinal centers (in primary follicles; Janeway et al., 2004); therefore, changes in the numbers of germinal centers and associated B-cells can reflect major responses to exposure to antigens or toxicants (Vieira & Rajewsky, 1990; Takahashi et al., 1998). A marked decrease in total B-cell counts was seen in the MXC + PARA-treated mice compared with that in MXC and PARA mice. Neither other combinational treatment had a similar significant effect. At the germinal center level, both MXC + PARA and PBO + MXC led to significant reductions in B-cell levels; PARA + PBO had no significant impact. Compared to their individual agents, MXC + PARA treatment caused even greater reductions in total B-cell levels, but had no effect at the germinal center level. This contrasts with PBO + MXC that had the opposite effect, i.e. no impact at total B-cell level but significantly-so at germinal centers. While these opposing outcomes are without explanation at this point, the upshot is that the combinational treatments with PBO + MXC or MXC + PARA are toxic to B-cells *in situ*. Toxicity from PARA + PBO is nominal at best.

The findings with the PBO + MXC mice supports our contention cited in the early paragraphs about potentially more selective effects on B-cells. That the MXC + PARA regimen also impacted on B-cells (beyond above-noted effects on thymic weights, T-cell counts, and IgM responses) suggested that this specific combination displayed a far more immunotoxic targeting than the other combined regimen. Whether such a divergent effect is due to differences in synergizing effects from each individual agent is an interesting possibility. Future studies with gradational combinations of each test chemical should allow us to ascertain which of the individual agents is driving any synergisms.

Conclusions

Our data show that combined exposure to certain environmental chemicals can induce immunotoxicity, as shown by effects on SRBC-specific IgM responses and T- or B-cell counts, compared to that by individual exposure to the chemicals in mixtures. However, this toxicity appears to differ, depending on which chemicals are combined. In particular, it was clear that, among the three combinations, MXC + PARA presented the most immunotoxic profile in the murine hosts. The combined toxicity may be affected by chemical structure, receptor binding, and immune pathways involved; further studies are currently in progress. It is expected that the results of this study will help others in their evaluation of immunotoxic combinational effects when conducting assessments of the safety of environmental/occupational chemicals.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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