IMMUNOLOGY

'Danger' signals

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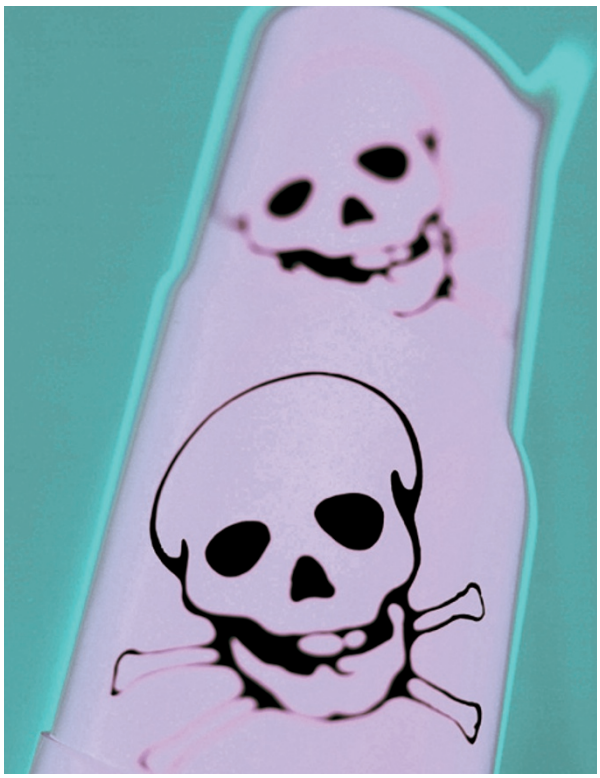
Toll-like receptors (TLRs) recognize exogenous and endogenous 'danger' signals, but how is an efficient immune response to dying tumour cells mounted? Guido Kroemer, Laurence Zitvogel and colleagues describe a previously unknown pathway by which tumour cells dying after treatment with chemotherapy are recognized by TLRs and trigger an immune response.

Inoculation of doxorubicin-treated or oxaliplatin-treated dying thymoma, sarcoma or colon cancer cells into the foot pad of mice wild-type for or lacking different TLRs showed that only *Tlr4*$^{-/-}$ mice were defective in T-cell priming (measured as interferon-γ production) after re-stimulation with tumour antigen. If dendritic cells (DCs) were depleted in wild-type mice, the priming of T cells by dying tumour cells was abrogated. Furthermore, when either wild-type or *Tlr4*$^{-/-}$ DCs were exposed to dying tumour cells and transferred into *Tlr4*$^{-/-}$ mice, only the DCs with no TLR4 failed to activate T cells, showing that TLR4^{+} DCs are required for the immune response. The authors then used co-precipitation assays to show that the endogenous high-mobility group box 1 protein (HMGB1) is released by dying tumour cells and constitutes a danger signal that mobilizes the immune response by binding and stimulating TLR4 on DCs. This signal is necessary because pre-incubation of tumour cells with a small interfering RNA (siRNA) or a neutralizing antibody against HMGB1 inhibited the capacity of dying tumour cells to stimulate DCs. After recognition of HMGB1, TLR4 transduces signals through the TLR adaptor myeloid differentiation primary response protein (MYD88), as *Myd88*$^{-/-}$ DCs behaved in the same way as *Tlr4*$^{-/-}$ DCs when exposed to dying tumour cells.



So, what role does the HMGB1–TLR4–MYD88 pathway have in the efficacy of anticancer drugs? *Tlr4*$^{-/-}$ mice, or dying tumour cells lacking HMGB1, could not trigger an effective anti-tumour response against the same tumour cells inoculated one week after the initial injection. If mice with established tumours lacked TLR4 or MYD88, chemotherapy or local radiotherapy was not as effective at reducing tumour growth or prolonging survival as in wild-type mice.

What relevance might these findings have for patients? 8–10% of Caucasians have a polymorphism in *TLR4* (Asp299Gly), which could compromise the effectiveness of chemotherapy in breast cancer. The authors found that this polymorphism reduced the interaction between TLR4 and HMGB1, and prevented DCs from presenting antigens from dying tumour cells to cytotoxic T cells. They analysed the time to metastasis in 280 patients with non-metastatic breast cancer who had been treated with anthracyclines after surgery because of lymph node involvement. The frequency of metastasis by 5 years after surgery was 40% in those with mutant TLR4, compared with 26.5% in patients with wild-type TLR4, and metastasis-free survival of patients with mutant TLR4 was also significantly lower.

Dying tumour cells therefore elicit an immune response that is required for the success of therapy, and could possibly be exploited to improve the immunogenicity of current chemotherapy regimens.

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ORIGINAL RESEARCH PAPER Apetoh, L. *et al.* Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Med.* 19 August 2007 (doi: 10.1038/nm1622)