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*Lupus* 2005 14: 417

DOI: 10.1191/0961203305lu2102rr

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## REVIEW

# A Toll for lupus

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Toll-like receptor (TLR)-9 recognizes CpG motifs in microbial DNA. TLR9 signalling stimulates innate antimicrobial immunity and modulates adaptive immune responses including autoimmunity against chromatin, e.g., in systemic lupus erythematosus (SLE). This review summarizes the available data for a role of TLR9 signalling in lupus and discusses the following questions that arise from these observations: 1) Is CpG-DNA/TLR9 interaction involved in infection-induced disease activity of lupus? 2) What are the risks of CpG motifs in vaccine adjuvants for lupus patients? 3) Is TLR9 signalling involved in the pathogenesis of lupus by recognizing self DNA? *Lupus* (2005) **14**, 417–422.

**Key words:** autoimmunity; DNA; infection; inflammation; kidney

## Introduction

Lupus represents a number of different pathophysiological and clinical syndromes that share autoimmunity against particles of self DNA. DNA autoimmunity develops either from genetic or yet undefined environmental factors, or a combination of both. The latter involves the general problem of each organism to keep the balance between tolerance to the self and to maintain immunity to the foreign. Lupus teaches us, that under normal conditions, tolerance to self DNA is maintained by a number of mechanisms. Loss of tolerance to self DNA occurs when genetic or external factors lead to a functional blockade of such mechanisms. In lupus, autoimmune tissue injury is thought to be mediated by immune complexes that contain nucleosomes or other chromatin-containing particles. These immune complexes are recognized through Fc receptors and subsequent complement activation. The immunostimulatory effects of the chromatin itself remained poorly defined.

The recognition of the cytosine-guanosine dinucleotide as the stimulatory motif in bacterial DNA, a decade ago,<sup>1</sup> has raised enormous research activities aiming to unravel the immunological properties of DNA sequence elements. This review provides an overview about the immunobiology of such DNA sequence elements and discusses several issues that link these data to the

pathogenesis of lupus (Table 1). Further questions that are discussed include: 1) Is CpG-DNA involved in infection-induced disease activity of lupus? 2) What are the risks of CpG motifs in vaccine adjuvants for lupus patients? 3) Is the recognition of CpG motifs in self DNA involved in the pathogenesis of lupus?

## CpG-DNA is an ancient pathogen-associated molecular pattern that causes immunity

DNA is traditionally thought to be a condensed medium of information that allows reproduction and that encodes for a certain phenotype. However, DNA has additional functions. Only a decade ago Arthur M Krieg in Dennis M Klinman's laboratory first described the cytosine-guanosine dinucleotide motif as the substrate of the immunostimulatory effects of bacterial DNA.<sup>1</sup> However, CpG motifs are present in DNAs of all kinds of microbes, better to say, in all species.<sup>2</sup> The discovery of other microbial ligands that serve as pathogen-associated molecular pattern (PAMPs) including lipopolysaccharides, peptidoglycans, RNAs or lipopeptides suggest that the recognition of PAMPs provide signals to the eukariont about its microbial environment very early during evolution.<sup>3</sup> Accumulating data show that among other microbial nucleic acids bacterial CpG-DNA is a potent activator of innate antimicrobial immunity and a modulator of adaptive immune responses. Bacterial CpG-DNA was first described as a B cell mitogen.<sup>1</sup> In addition, immune complexes that contain unmethylated CpG-DNA, have been demonstrated to induce T cell-independent

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Received 1 October 2004; accepted 9 November 2004

**Table 1** Mechanisms that link CpG-DNA to the pathogenesis of lupus

<i>B cells</i>	
Bacterial DNA is a B cell mitogen	Ref. 1
CpG motifs in chromatin particles crosslink the BCR to TLR9 which leads to T cell independent B cell proliferation and autoantibody production	Ref. 4
<i>Dendritic cells</i>	
CpG-DNA induces dendritic cells to produce Th1 cytokines	Ref. 6
CpG-DNA can induce dendritic cells to produce IL-6	Ref. 12
<i>T cells</i>	
Known triggers of SLE lead to hypomethylation of CpG motifs in self DNA	Ref. 35
IL-6 blocks the inhibitory function of regulatory T cells	Ref. 12

activation, proliferation, and autoantibody production by B-cells.<sup>4</sup> In fact, covalent linkage of CpG-DNA to an antigen provides a powerful signal for the activation of other antigen-presenting cells, including dendritic cells and, in mice, macrophages.<sup>5</sup> Exposure to CpG-DNA induces antigen-presenting cells to secrete Th1 cytokines, i.e., IL-12 or IFN- $\gamma$ .<sup>6</sup> Subsequent T cell or B cell responses are modulated towards a Th1-type response which can be therapeutic in Th2-type diseases like asthma and atopy<sup>7</sup> and that provide the rationale for using CpG DNA as a vaccine adjuvant.<sup>8</sup> However, a number of studies suggest that the immunostimulatory effects of CpG-DNA can initiate or unfavourably modulate autoimmunity (Table 1). For example, exposure to CpG-DNA aggravates various types of chronic autoimmune tissue injury, e.g., arthritis, encephalitis or glomerulonephritis.<sup>9–11</sup> Furthermore, CpG-DNA-induced secretion of IL-6 by dendritic cells blocks the inhibitory effect of regulatory T cells leading to uncontrolled proliferation of autoreactive T cells.<sup>12</sup> This mechanism may have important implications on the role of CpG-DNA in autoimmunity.<sup>13</sup> At high doses CpG-DNA-induced activation of TLR9 can lead to toxic shock.<sup>14</sup> Repeated injections with CpG-DNA cause liver cell apoptosis, systemic cytokine release, lymphoproliferation, and functional immunosuppression.<sup>15</sup> Thus, CpG-DNA has potent immunomodulatory functions, that may well be involved in the pathogenesis of lupus.

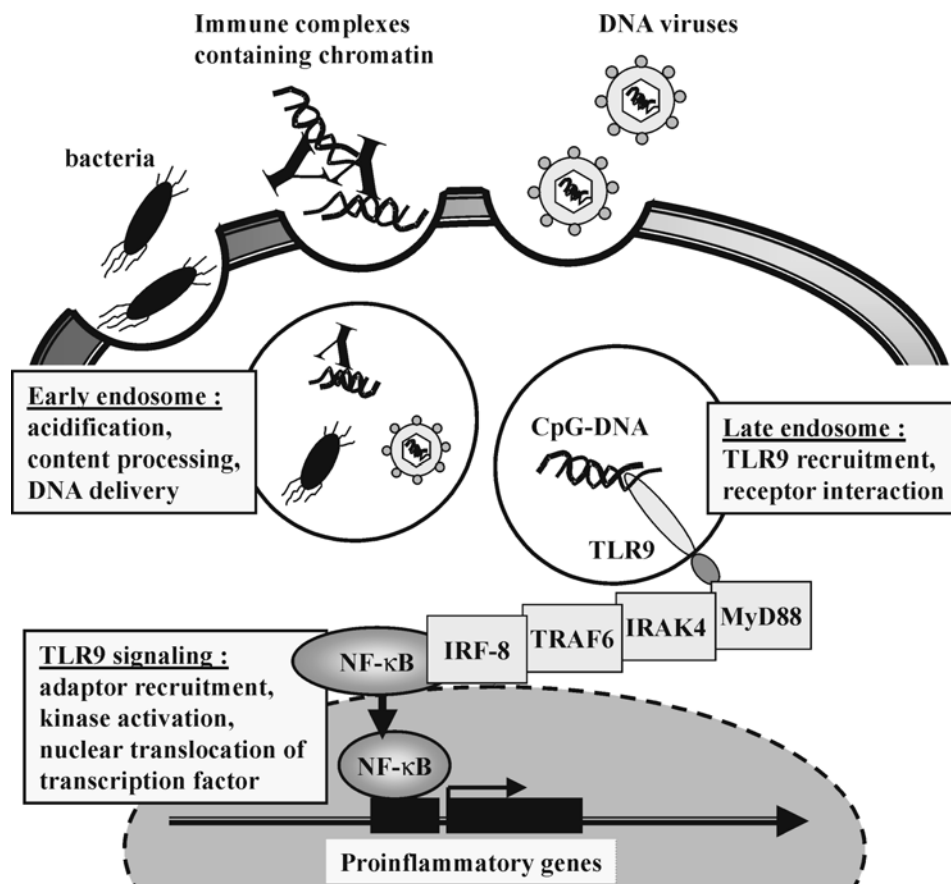
### CpG-DNA signals through Toll-like receptor (TLR)-9

PAMPs need to be recognized by appropriate pattern recognition receptors. In the year 2000, Shizuo Akira's group identified a Toll-like receptor, TLR9, to exclusively recognize CpG-DNA.<sup>14</sup> TLR9-deficient mice did not respond to CpG-DNA and cells isolated

from these mice remained unresponsive upon stimulation with CpG-DNA *in vitro*.<sup>14</sup> TLR9 belongs to the subgroup of nucleic acid-specific TLRs that also include TLR3 (ligand: double-stranded viral RNA) and TLR7/8 (ligand: single-stranded viral RNA) which share genetic and biological homologies. TLR9 turned out to be expressed only by a small set of immune cells. In humans, TLR9 expression is restricted to B cells and to plasmacytoid dendritic cells, whereas in mice TLR9 is expressed on additional antigen presenting cells including monocyte/macrophages and myeloid dendritic cells.<sup>5</sup> In these cells TLR9 is located in the endoplasmic reticulum.<sup>16</sup> After uptake extracellular material is processed in endosomes which induces TLR9 redistribution from the endoplasmic reticulum to late endosomes.<sup>16</sup> Interestingly, the stimulatory effect of CpG-DNA on the cellular uptake of CpG-DNA-conjugates is independent of TLR9, suggesting another yet undefined DNA receptor.<sup>17</sup> CpG-oligodeoxyribonucleotides (ODN) form aggregates that allow direct binding to TLR9, a process that is pH dependent,<sup>18–20</sup> as chloroquine and other inhibitors of endosomal acidification are potent inhibitors of TLR9 activation by CpG-DNA.<sup>5,19</sup> This finding possibly represents the previously unknown molecular mechanism for the efficacy of chloroquine treatment in lupus. The interaction of TLR9 with CpG-DNA leads to recruitment of the adaptor protein myeloid differentiation primary-response protein 88 (MyD88) from the cytosol to the outer endosomal membrane where it interacts with the intracellular Toll-interleukin 1 receptor (TIR) domain of TLR9 (Figure 1). Through activating interleukin-1 receptor-associated kinase (IRAK)-4, TNF receptor associated factor (TRAF)-6, and interferon regulatory factor-8/IFN consensus sequence binding protein TLR9 signalling finally activates NF- $\kappa$ B.<sup>9,21,22</sup> After translocation into the nucleus NF- $\kappa$ B induces the expression of many proinflammatory genes including TNF $\alpha$ , IL-6, IL-1 $\beta$ , IL-8, IL-12, IL-18, as well as CC- and CXC-chemokines.<sup>23</sup> The fact that different classes of CpG-ODN have been shown to cause different immunostimulatory activities suggests additional yet unknown modulators of TLR9-induced signal transduction.<sup>24,25</sup>

### CpG-DNA/TLR9 interaction modulates innate and adaptive immunity in lupus

The potential of CpG-DNA for activating B cells and plasmacytoid dendritic cells suggest that TLR9 might play a role in the pathogenesis of lupus.<sup>26,27</sup> In fact,



**Figure 1** DNA recognition through TLR-9. TLR9 is located in the ER and merges to intracellular endosomes upon uptake of microorganisms or immune complexes. TLR9 signals exclusively through the MyD88–IRAK4–TRAF6–IRF-8 pathway and subsequently induces nuclear translocation of NF-κB. NF-κB-dependent gene expression includes many proinflammatory mediators, e.g., cytokines and chemokines.

B cells isolated from MRL<sup>lpr/lpr</sup> mice with lupus-like disease, produce large amounts of autoantibodies when exposed to immune complexes that contain CpG-DNA.<sup>4</sup> Autoreactive B-cells from these mice recognize the IgG part of the immune complex by their surface BCR, then internalize the immune complex which exposes CpG-DNA to TLR9 in the endosomal compartment.<sup>28</sup> These mechanisms may also apply *in vivo*, because autoimmune MRL<sup>lpr/lpr</sup> mice injected with bacterial CpG-DNA produce large amounts of DNA autoantibodies<sup>29</sup> in association with increased MHC II expression on B cells isolated from spleens of these mice (unpublished observation). The avidity of DNA autoantibodies present in MRL<sup>lpr/lpr</sup> mice and those induced by unmethylated CpG-DNA is comparable.<sup>30</sup> Systemic exposure to bacterial CpG-DNA can induce the production of DNA autoantibodies in nonimmune mice.<sup>31</sup> Furthermore, CpG-DNA has a strong adjuvant effect on DNA autoantibody production in mice that have been exposed to vertebrate DNA.<sup>31</sup> These data support the hypothesis that in systemic lupus erythematosus (SLE) exposure to

bacterial DNA, e.g., during bacterial infection, provides a strong signal for enhanced DNA autoantibody production. The presence of unmethylated CpG-DNA in immune complexes facilitate their intracellular uptake and exposure to TLR9 in endosomes via IgG/BCR interaction in B cells.<sup>28,32</sup>

As outlined above, TLR9 is expressed in additional leukocyte subsets which may cause other immune effects in addition to B cell activation after exposure to CpG-DNA in SLE. Thus, we determined the distribution of rhodamin-labelled CpG-DNA after injection into 16-weeks old autoimmune MRL<sup>lpr/lpr</sup> mice and nonautoimmune MRL wild-type mice.<sup>29</sup> In MRL<sup>lpr/lpr</sup> mice CpG-DNA localized to infiltrating F4/80 positive macrophages and CD11c positive dendritic cells in the glomerular and tubulointerstitial compartment of nephritic kidneys. By contrast, in healthy MRL wild-type mice CpG-DNA did not localize to the kidney. The distribution of CpG-DNA was consistent with TLR9 protein expression in monocytic inflammatory cell infiltrates upon immunostaining. MRL<sup>lpr/lpr</sup> mice treated with CpG-DNA every other day from

week 16 to 18 of age showed marked increase in renal expression of NF- $\kappa$ B-dependent CC-chemokines which localized to areas of TLR9 positive cells. Thus, systemic exposure to bacterial CpG-DNA involves activation of TLR9 positive cells in preexisting inflammatory cell infiltrates, e.g., in lupus nephritis. Enhanced DNA autoantibody production and activation of tissue monocytes, occurred in parallel and this was associated with severe aggravation of lupus nephritis.<sup>29</sup> These data support the hypothesis that in SLE intercurrent bacterial infections can trigger disease activity by circulating bacterial CpG-DNA via TLR9 on autoreactive B cells and selected tissue monocyte populations. In the latter TLR9 activation can add on the TLR9-independent stimulation that is provided by the immune complex itself.<sup>33</sup>

Infections might aggravate lupus also through other TLRs. In fact, in a similar experimental set up we found that viral double-stranded RNA induced marked autoimmune disease activity in MRL<sup>lpr/lpr</sup> mice (manuscript in revision). However, this was mediated by different mechanisms, because TLR3 has a different expression profile as compared to TLR9. For example, B cells lack TLR3 and double-stranded RNA fails to activate B cells and DNA autoantibody production in MRL<sup>lpr/lpr</sup> mice. In contrast, TLR3 is expressed on glomerular mesangial cells and double-stranded RNA induces mesangial cells to produce cytokines and chemokines, known to aggravate glomerular inflammation (unpublished data).

### Does TLR9 recognize self-DNA?

Chromatin-containing particles are major antigens in lupus. Thus, the question arises whether TLR9-related recognition of CpG motifs in self DNA is involved in the pathogenesis of SLE? From the early times of evolution a Toll receptor that recognizes microbial DNA required mechanisms that prevent self DNA recognition in order to avoid autoimmunity. In vertebrates three such mechanisms have been identified. These include the methylation state of CpG motifs, the frequency of CpG motifs, and inhibitory DNA sequences.

#### *Methylation state of CpG motifs*

Microbial DNA is unmethylated as microbes lack methyltransferases. Methylation reduces the immunostimulatory effects of bacterial DNA<sup>34</sup> and treatment with CpG methylase inactivates CpG-ODN.<sup>1</sup> In vertebrates CpG motif cytosines are 70–80% methylated which is thought to contribute to the lack of immunostimulatory activity of their DNA.<sup>35</sup> In fact, hypomethylation of self DNA can be associated with

autoimmunity and lupus,<sup>36</sup> indicating that hypomethylated CpG motifs in self DNA may contribute to the pathogenesis of lupus. For example, inhibiting DNA methylation in mature T cells induces LFA-1 positive autoreactive T cells that mediate DNA autoantibody production.<sup>37</sup> Known triggers of lupus-like syndromes including UV light, hydralazine, and procainamide inhibit the activity of DNA methyltransferases and induce autoreactive T cell subsets.<sup>37</sup> Patients with active lupus have decreased enzyme activity of DNA methyltransferases, lower rates of genomic methylated cytosine nucleotides, and increased levels of circulating hypomethylated DNA.<sup>38</sup> Patients with active lupus also have increased numbers of autoreactive T cells that overexpress LFA-1.<sup>38</sup> These data support the hypothesis that DNA methylation provides tolerance to self DNA and that impaired DNA methylation can be associated with lupus disease activity, possibly through the recognition of CpG motifs via TLR9.

#### *Frequency of CpG-motifs*

Vertebrate DNA that has been completely demethylated still shows only weak stimulatory activity as compared to bacterial DNA.<sup>39</sup> These data argue for additional factors that protect self-DNA from activating immunity. Several studies have addressed this issue by comparative genome analysis for the frequency of CpG-motifs in different species. For example, stimulatory CpG motifs are present in vertebrate genomes but at only a frequency of 20% of random frequency.<sup>40</sup> By contrast, CpG motifs are overrepresented in *E. coli* DNA<sup>39</sup> supporting the idea that during evolution of species stimulatory CpG motifs were negatively selected in vertebrate DNAs and positively selected in bacterial DNA. This would explain why similar amounts of unmethylated bacterial DNA activate TLR9 positive human cells to a greater extent than demethylated human DNA.

#### *Inhibitory DNA sequences*

In their search for ODN that have optimal stimulatory activity several groups detected sequence motifs that inhibit CpG-DNA-induced effects. Screening the mouse and *E. coli* genomes for the frequency of such inhibitory DNA sequences showed that such inhibitory DNA sequence elements are present at a high frequency in the mouse genome and underrepresented in the *E. coli* genome.<sup>39</sup> Obviously, the ratio of stimulatory and inhibitory sequence elements regulates the immunomodulatory potential of DNA. Following this hypothesis human self DNA that includes a high number of inhibitory sequence elements would neutralize

the small number of (methylated) CpG motifs. In view of TLR9 recognizing CpG motifs this would represent another mechanism to discriminate self DNA from microbial DNA, the latter characterized by a high number of (unmethylated) CpG motifs in the presence of a small number of inhibitory sequences.

### Stimulatory and inhibitory DNA sequence motifs can modulate immunity

ODNs that include either stimulatory, inhibitory or inert sequence elements may therefore represent an interesting tool for studying the immunomodulatory functions of DNA *in vitro* and *in vivo* but may also provide new questions for patients with lupus. 1) Will therapeutic use of CpG-DNA for other indications cause disease activity in SLE patients? 2) Does CpG-DNA therapy cause drug-induced lupus? 3) Can we treat lupus with ODN containing inhibitory sequence elements?

#### *Safety of CpG-ODN therapy in SLE*

CpG-ODN are currently in clinical trials for the treatment of cancer, atopy and as vaccine adjuvants.<sup>8,41,42</sup> Our findings with CpG-ODN in murine SLE raise considerable concern about the safety of CpG-ODN therapy in SLE patients.<sup>29</sup> Exposure to unmethylated immunostimulatory CpG-ODN may cause similar aggravation of disease in SLE patients as CpG-ODN in autoimmune MRL<sup>lpr/lpr</sup> mice. These data are supported by an increasing number of reports showing that CpG-ODN can aggravate other animal models of chronic autoimmune tissue injury, e.g., arthritis, encephalitis or glomerulonephritis.<sup>9–11</sup> However, toxicity may be related to the dose, treatment intervals, and the route of administration of CpG-ODN. All experimental data showing disease aggravation are derived from studies that used repeated intraperitoneal injections of CpG-ODN. Topical application or single vaccination regimen might have less effects on preexisting autoimmunity.

#### *Drug-induced lupus*

The ability of bacterial DNA to induce DNA autoantibodies *in vitro* and *in vivo* suggests that CpG-ODN therapy might trigger drug-induced lupus. Clinical or serological signs of drug-induced lupus did not occur in a recently reported trials that applied CpG-DNA as a vaccine adjuvant.<sup>41,43</sup> However, in view of the history of the reported induction of DNA autoantibodies with anti-TNF $\alpha$  treatment regimen that increased from 'never observed' in phase I trials to

56% in more recent studies,<sup>44</sup> one should await studies with higher numbers of patients before a firm statement upon the potency of CpG-ODN to induce lupus-like syndromes can be made.

#### *Treating lupus with inhibitory DNA*

As outlined above inhibitory DNA sequences may suppress the stimulatory effects of CpG motifs in human self DNA. Based on *in vitro* studies showing that CpG-induced immunity can be blocked with ODN containing inhibitory DNA motifs the hypothesis of targeting SLE with such ODN evolves. We have tested this hypothesis and found that inhibitory ODN can effectively block CpG-ODN-induced effects *in vivo* (unpublished observation). Whether the onset or progression of spontaneous SLE can be prevented by injections of inhibitory ODN is currently under study. Only the latter would argue for a role of TLR9 in the pathogenesis of lupus. In that case developing specific small molecule TLR9 antagonists would represent an interesting perspective for the treatment of SLE.

### Summary

Lupus is characterized by autoimmunity against self DNA. The recognition of TLR9 as an ancient receptor that recognizes unmethylated CpG-DNA raises a set of questions of its role in the pathogenesis of SLE. At present there is evidence that TLR9 signaling is involved in the lupus disease activity that is triggered by unmethylated microbial DNA, e.g., during intercurrent microbial infections that trigger lupus flares. In contrast to microbial DNA, CpG motifs in self DNA are suppressed by several mechanisms, which provide tolerance to self DNA under normal conditions. Whether lupus pathogenesis involves TLR9-dependent recognition of hypomethylated self DNA is still unclear, although a number of studies provide data that support this hypothesis. If impaired tolerance to self DNA will turn out to involve TLR9, then TLR9 may become an interesting target for therapeutic intervention in lupus.

### Acknowledgement

The work was supported by a grant from the Deutsche Forschungsgemeinschaft (AN372/4-1) and the Fritz Thyssen Foundation to H-JA.

## References

- 1 Krieg AM, Yi AK, Matson S *et al*. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 1995; **374**: 546–549.
- 2 Hendrich B, Tweedie S. The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends Genet* 2003; **19**: 269–277.
- 3 Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol* 2003; **21**: 335–376.
- 4 Leadbetter EA, Rifkin IR, Hohlbaum AM *et al*. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* 2002; **416**: 603–607.
- 5 Wagner H. The immunobiology of the TLR9 subfamily. *Trends Immunol* 2004; **25**: 381–386.
- 6 Heeg K, Zimmermann S. CpG DNA as a Th1 trigger. *Int Arch Allergy Immunol* 2000; **121**: 87–97.
- 7 Walker C, Zuany-Amorim C. New trends in immunotherapy to prevent atopic diseases. *Trends Pharmacol Sci* 2001; **22**: 84–90.
- 8 Ulevitch RJ. Therapeutics targeting the innate immune system. *Nat Rev Immunol* 2004; **4**: 512–520.
- 9 Anders HJ, Banas B, Linde Y *et al*. Bacterial CpG-DNA aggravates immune complex glomerulonephritis: role of TLR9-mediated expression of chemokines and chemokine receptors. *J Am Soc Nephrol* 2003; **14**: 317–326.
- 10 Ichikawa HT, Williams LP, Segal BM. Activation of APCs through CD40 or Toll-like receptor 9 overcomes tolerance and precipitates autoimmune disease. *J Immunol* 2002; **169**: 2781–2787.
- 11 Miyata M, Kobayashi H, Sasajima T, Sato Y, Kasukawa R. Unmethylated oligo-DNA containing CpG motifs aggravates collagen-induced arthritis in mice. *Arthritis Rheum* 2000; **43**: 2578–2582.
- 12 Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4 + CD25 + T cell-mediated suppression by dendritic cells. *Science* 2003; **299**: 1033–1036.
- 13 Pasare C, Medzhitov R. Toll-like receptors: balancing host resistance with immune tolerance. *Curr Opin Immunol* 2003; **15**: 677–682.
- 14 Hemmi H, Takeuchi O, Kawai T *et al*. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000; **408**: 740–745.
- 15 Heikenwalder M, Polymenidou M, Junt T *et al*. Lymphoid follicle destruction and immunosuppression after repeated CpG oligodeoxynucleotide administration. *Nat Med* 2004; **10**: 187–192.
- 16 Latz E, Schoenemeyer A, Visintin A *et al*. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol* 2004; **5**: 190–198.
- 17 Heit A, Maurer T, Hochrein H *et al*. Cutting edge: Toll-like receptor 9 expression is not required for CpG DNA-aided cross-presentation of DNA-conjugated antigens but essential for cross-priming of CD8 T cells. *J Immunol* 2003; **170**: 2802–2805.
- 18 Cornelie S, Hoebeke J, Schacht AM *et al*. Direct evidence that toll-like receptor 9 (TLR9) functionally binds plasmid DNA by specific cytosine-phosphate-guanine motif recognition. *J Biol Chem* 2004; **279**: 15124–15129.
- 19 Rutz M, Metzger J, Gellert T *et al*. Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. *Eur J Immunol* 2004; **34**: 2541–2550.
- 20 Wu CC, Lee J, Raz E, Corr M, Carson DA. Necessity of oligonucleotide aggregation for toll-like receptor 9 activation. *J Biol Chem* 2004; **279**: 33071–33078.
- 21 Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; **4**: 499–511.
- 22 Tsujimura H, Tamura T, Kong HJ *et al*. Toll-like receptor 9 signaling activates NF-kappaB through IFN regulatory factor-8/IFN consensus sequence binding protein in dendritic cells. *J Immunol* 2004; **172**: 6820–6827.
- 23 Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. *Nat Rev Mol Cell Biol* 2004; **5**: 392–401.
- 24 Kerkmann M, Rothenfusser S, Hornung V *et al*. Activation with CpG-A and CpG-B oligonucleotides reveals two distinct regulatory pathways of type I IFN synthesis in human plasmacytoid dendritic cells. *J Immunol* 2003; **170**: 4465–4474.
- 25 Vollmer J, Weeratna R, Payette P *et al*. Characterization of three CpG oligodeoxynucleotide classes with distinct activities. *Eur J Immunol* 2004; **34**: 251–262.
- 26 Krieg AM. A role for Toll in autoimmunity. *Nat Immunol* 2002; **3**: 423–424.
- 27 Vinuesa CG, Goodnow CC. Immunology: DNA drives autoimmunity. *Nature* 2002; **416**: 595–598.
- 28 Viglianti GA, Lau CM, Hanley TM, Miko BA, Shlomchik MJ, Marshak-Rothstein A. Activation of autoreactive B cells by CpG dsDNA. *Immunity* 2003; **19**: 837–847.
- 29 Anders HJ, Vielhauer V, Eis V *et al*. Activation of toll-like receptor-9 induces progression of renal disease in MRL-Fas(lpr) mice. *FASEB J* 2004; **18**: 534–536.
- 30 Tran TT, Reich CF 3rd, Alam M, Pisetsky DS. Specificity and immunochemical properties of anti-DNA antibodies induced in normal mice by immunization with mammalian DNA with a CpG oligonucleotide as adjuvant. *Clin Immunol* 2003; **109**: 278–287.
- 31 Pisetsky DS, Wenk KS, Reich CF 3rd. The role of cpg sequences in the induction of anti-DNA antibodies. *Clin Immunol* 2001; **100**: 157–163.
- 32 Leadbetter EA, Rifkin IR, Marshak-Rothstein A. Toll-like receptors and activation of autoreactive B cells. *Curr Dir Autoimmun* 2003; **6**: 105–122.
- 33 Boule MW, Broughton C, Mackay F, Akira S, Marshak-Rothstein A, Rifkin IR. Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. *J Exp Med* 2004; **199**: 1631–1640.
- 34 Stacey KJ, Sweet MJ, Hume DA. Macrophages ingest and are activated by bacterial DNA. *J Immunol* 1996; **157**: 2116–2124.
- 35 Bird AP. CpG-rich islands and the function of DNA methylation. *Nature* 1986; **321**: 209–213.
- 36 Yung RL, Richardson BC. Role of T cell DNA methylation in lupus syndromes. *Lupus* 1994; **3**: 487–491.
- 37 Richardson B. DNA methylation and autoimmune disease. *Clin Immunol* 2003; **109**: 72–79.
- 38 Richardson BC, Strahler JR, Pivrotto TS *et al*. Phenotypic and functional similarities between 5-azacytidine-treated T cells and a T cell subset in patients with active systemic lupus erythematosus. *Arthritis Rheum* 1992; **35**: 647–662.
- 39 Stacey KJ, Young GR, Clark F *et al*. The molecular basis for the lack of immunostimulatory activity of vertebrate DNA. *J Immunol* 2003; **170**: 3614–3620.
- 40 Bird AP. CpG islands as gene markers in the vertebrate nucleus. *Trends Genet* 1987; **3**: 342–347.
- 41 Halperin SA, Van Nest G, Smith B, Abtahi S, Whiley H, Eiden JJ. A phase I study of the safety and immunogenicity of recombinant hepatitis B surface antigen co-administered with an immunostimulatory phosphorothioate oligonucleotide adjuvant. *Vaccine* 2003; **21**: 2461–2467.
- 42 Krieg AM. From bugs to drugs: therapeutic immunomodulation with oligodeoxynucleotides containing CpG sequences from bacterial DNA. *Antisense Nucleic Acid Drug Dev* 2001; **11**: 181–188.
- 43 Cooper CL, Davis HL, Morris ML *et al*. Safety and immunogenicity of CPG 7909 injection as an adjuvant to Fluarix influenza vaccine. *Vaccine* 2004; **22**: 3136–3143.
- 44 Vermeire S, Noman M, Van Assche G *et al*. Autoimmunity associated with anti-tumor necrosis factor alpha treatment in Crohn's disease: a prospective cohort study. *Gastroenterology* 2003; **125**: 32–39.