Differential regulation of antigen-specific IgG4 and IgE antibodies in response to recombinant filarial proteins

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Abstract

Having identified two recombinant filarial proteins (Ov27 and OvD5B) that induced patient peripheral blood mononuclear cells to produce antigen-specific IgG4/IgE antibodies in vitro, we assessed the role these filarial antigens play in inducing antigen-specific isotype switching (γ4 and ε) in the absence of T cells. Purified CD19+ sy/β2 se− B cells were cultured with either of these antigens in the presence of anti-CD40 mAb and human IL-4. Both antigen and polyclonal signals delivered by IL-4 (or IL-13) were necessary for the induction of specific IgG4/IgE antibodies. To assess the role played by cytokines produced by B lymphocytes in antigen-driven selection of the γ4 or ε isotype, neutralizing anti-cytokine antibodies were used in vitro. While anti-IL-12 antibodies did not alter the antigen-specific IgG4/IgE production, anti-IL-6, anti-IL-13 and anti-tumor necrosis factor-α antibodies significantly inhibited the production of IgG4/IgE. Anti-IL-2 and anti-IL-10 antibodies appeared to down-regulate antigen-specific IgG4 antibodies without affecting antigen-specific IgE antibodies. Although anti-CD21 antibodies had no effect on specific IgE antibodies, they up-regulated specific IgG4 antibodies, a finding paralleled by anti-CD23 antibodies. These data suggest that certain filarial antigen-specific IgG4/IgE responses can be differentially regulated and that certain endogenously produced molecules from B cells—such as IL-2, IL-10, CD23 and CD21—play a significant role in the induction of specific isotypes of antigen-specific antibodies.

Introduction

Parasitic helminth infections in humans are commonly associated with blood and tissue eosinophilia along with elevated levels of serum IgG4 and IgE antibodies. The factors responsible for this immunological profile reflect the expansion of Th2-type CD4+ T cells capable of producing, among others, IL-5, IL-4 and IL-13, the latter two being implicated in IgG4 and IgE antibody production (1). Nevertheless, it is not fully understood why clinically apparent immediate hypersensitivity reactions are not frequent in helminth infections, in contrast to atopic disorders that share many of these immunological features (2). One possible explanation for the regulation of allergic-like reactivity is the presence of high levels of blocking IgG4 antigens (3). While the factors involved in the regulation of antigen-specific blocking antibodies have not been fully elucidated, the suggestion has been raised that it is the chronic antigenic exposure to antigens that is important in causing B lymphocytes from individuals chronically exposed to helminths to switch to the production of IgG4 and IgE.

In a previous study (4), by screening a panel of recombinant filarial antigens, two recombinant filarial antigens, Ov27/cystatin and OvD5B, were identified as being able to drive peripheral blood mononuclear cells (PBMC) from sensitized individuals to produce IgG4 and IgE. The current study utilized an in vitro system in which purified CD19+ sy/β2 se− B cells from filaria-infected patients are stimulated with anti-CD40 mAb, human IL-4 and recombinant filarial antigens to induce specific IgG4 and IgE antibodies. The data suggest that three signals (two T cell derived and one from the antigen)
are required to induce switching to IgG4 and IgE, and that B cell-derived cytokines are critical for the switching to occur efficiently.

Methods

Cell preparations

Cryopreserved PBMC were obtained from individuals with Loa loa or Onchocerca volvulus as described previously (4). PBMC were thawed, washed and cultured as described (4). B cells were recovered from PBMC using positive selection by means of CD19 (clone AB1)-coated magnetic beads (Dynabeads; Dynal, Lake Success, NY) according to the manufacturer’s instructions. CD19+ B cells were allowed to cap and shed antigen/antibody and bead complexes during an overnight incubation. In some experiments, the magnetic beads were detached from B cells using a competitive mAb (CD19-Detachabeads; Dynal), according to the recommendations of the manufacturer. CD19+ B cells were obtained from Detachabead-treated B cells using negative selection; B cells (10^6/ml) were incubated for 1 h at 4°C with 5 μg/ml of mouse mAb anti-human IgG (Jackson ImmunoResearch, West Grove, PA) and anti-human IgE (E25) (a gift from Dr P. Jardieu, Genentech, San Francisco, CA) followed by two cycles of incubation with magnetic goat anti-mouse antibody-coated beads (Dynabeads). Purification was assessed by cytofluorometry using phycoerythrin-conjugated anti-CD19 (leu-7; clone 4G7) mAb (Becton Dickinson, San Jose, CA) and with 2 μg/ml of mouse anti-IgG (HP6023) (Hybridoma Reagent, Kingsville, MD) and mouse anti-IgE antibody 7.12 (a gift from Dr A. Saxon, UCLA, Los Angeles, CA), or mouse mAb control, followed by an additional 30 min incubation (4°C) with FITC-conjugated goat anti-mouse antibody (Caltag, San Francisco, CA); the B cells were >98% CD19+ sIgE− slgG−.

Recombinant parasite proteins

The purified recombinant parasite proteins used in this study were obtained as maltose-binding fusion proteins (MBP). They were OvD5B (F. Perler, New England Biolabs, Beverly, MA), a putative aspartyl protease inhibitor with homology to Ov7 (GenBank X13313) and Ov27 (GenBank M37105) (J. Bradley, Imperial College of Science, Technology and Medicine, London, UK), a cysteine protease inhibitor analogous to Ov7/ cystatin. To assess the specificity of the response, another recombinant filarial antigen, OvGalBP (5), was also used.

In vitro antibody production

PBMC were adjusted to 2 × 10^6 cells/ml and CD19+ B cells to 10^6 cells/ml. The cells were cultured in 48-well plates (Costar, Cambridge, MA) at a final volume of 0.5 ml of Iscove’s Dulbecco’s modified medium (Biofluids, Rockville, MD) supplemented with 1% l-glutamine (Biofluids), 80 μg/ml gentamicin (BioWhittaker, Walkersville, MD), 1% insulin/transferrin/ selenium medium (Biofluids) and 10% FCS (Hyclone, Logan, UT). PBMC or B cell suspensions were cultured with recombinant parasite proteins (at optimal concentrations) and with the relevant MBP as a control. CD19+ B cells were cultured in the presence of the mitogenic anti-CD40 mAb 89 (10 ng/ml) (a gift from Dr J. Banchereau, Schering-Plough, Dardilly, France) with or without human IL-4 (5 ng/ml; obtained from CHO-transfected cell cultures). In some experiments, cultures were performed in the presence of recombinant human cytokines or neutralizing antibodies to human cytokines: IL-13 or TNF-α (5 ng/ml) (Peprotech, Rocky Hill, NJ); mouse anti-CD23 mAb (clone 9F25) (Amac, Westbrook, ME) and anti-CD21 mAb (clone BU-33, The Binding Site, San Diego, CA) were used at 10 ng/ml; neutralizing rat antihuman IL-10 (PharMingen, San Diego, CA) and rabbit antihuman IL-6 and anti-tumor necrosis factor (TNF)-α (Peprotech) were used at 10 μg/ml; neutralizing mouse anti-IL-2 (R & D Systems, Minneapolis, MN) and anti-IL-12 mAb (a combination of mAb to p70 (clone C8.6.2.2) and p40 (clone C11.79); gifts from Dr G. Trinchieri, The Wistar Institute, Philadelphia, PA) were used at 10 ng/ml; native (endotoxin-free) soluble CD23 (sCD23), a gift from Dr M. Sarfati (Hôpital Notre-Dame, Montreal, Canada), was used at 10 ng/ml. Control rabbit, rat and mouse anti-IgA (with irrelevant antigen specificities) were used where appropriate. Cultures were carried out at 37°C with 5% CO₂. Supernatants were collected at day 14 and frozen until assayed.

ELISA for antigen-specific IgG subclasses and IgE

Antigen-specific IgG4 and IgE antibodies were detected by ELISA as previously described using 0.5 μg/ml recombinant parasite protein or control fusion protein in NaHCO3/Na2CO3, pH 9.6, buffer (4°C). Data are expressed as OD ratios (recombinant protein OD values/MBP OD values).

Statistical analysis

Statistical analysis was performed using either the Mann-Whitney U (non-paired) or the Wilcoxon (paired) test.

Results

OvD5B and Ov27 induce filaria-infected patient CD19+ sIgE− slgG− B cells to produce antigen-specific IgG4 and/or IgE antibodies

In a series of 24 individual cell culture experiments, antigen (OvD5B or Ov27)-specific IgG4 and IgE production in PBMC cultures was compared with the production in CD19+ B cells from the same patients in which the B cells were also stimulated with anti-CD40 mAb and IL-4 as surrogates for T cell factors. When the culture supernatants were analyzed using specific IgG4 and IgE ELISAs, 14 of 24 produced IgG4 and/or IgE antibodies to at least one antigen (data not shown), whereas 100% of the individual patients’ separated B cells were capable of producing specific IgG4/IgE antibodies to either of the two antigens (data not shown). To test for the specificity of these responses, two additional studies were performed. First, these two recombinant antigens were added to B cells of normal, uninfected individuals and shown to be incapable of driving specific IgG4 and IgE. Second, another recombinant filarial antigen, OvGalBP, was used and shown not to be able to drive B cells from filaria-infected patients to produce specific IgG4 or IgE.

When IgG4/IgE production in the presence of antigen, anti-CD40 mAb, IL-4, and IL-13 was compared between
Fig. 1. Ov27- and OvD5B-induced specific IgG4/IgE antibody production does not result from the expansion of committed B cells. CD19+ B cells and B cells depleted of surface γ and ε (γε−) (n = 8) were exposed to antigens, anti-CD40 antibodies and IL-4 + IL-13 to produce antigen-specific IgG4 and IgE antibodies. Data are expressed as OD ratios (antigen-stimulated individual cultures divided by MBP control).

unfractionated CD19+ B cells and CD19+ B cells from which γ+ and ε+ cells were removed (>98% CD19+ se− γ−), there were no significant differences in the levels of antigen-specific IgG4 and IgE antibodies measured, suggesting that preswitched (γε− or se−) patient B cells were not responsible for the production of these specific antibodies (Fig. 1).

IL-4 is required for the production of antigen-induced IgG4/IgE antibodies by anti-CD40-stimulated CD19+ patient B cells

To assess the specificity of the antibody response and the role of IL-4 in the induction of specific IgG4/IgE antibody production, purified CD19+ B cells were stimulated with anti-CD40 mAb and IL-4 in the presence or absence of antigen (Table 1). As can be seen, IL-4 by itself was not responsible for causing the production of antigen-specific IgG4/IgE, as no specific antibody was produced in the presence of MBP, whereas with either antigen, specific IgG4 and IgE was induced and at levels that ranged from 5- to 50-fold above that seen in the control cultures.

In the presence of both IL-4 and antigen, specific IgG4/IgE was produced (P < 0.001 versus antigen only) (Fig. 2). Addition of IL-13 or TNF-α (not shown) to IL-4-containing cultures did not generally augment antigen-specific antibody production.

Certain cytokines and IgE-binding molecules differentially regulate the isotype and the antigen specificity of antibody production in response to Ov27 or OvD5B

To assess the role of endogenously produced cytokines in antigen-driven IgG4/IgE antibody production, CD19+ B cells from filarial-infected patients were stimulated with either Ov27 or OvD5B in the presence of anti-CD40 and IL-4 with or without neutralizing antibodies to a variety of cytokines, most having been described to be secreted by activated B cells (Fig. 3). Anti-IL-6, anti-TNF-α and anti-IL-13 antibodies consistently inhibited specific antibody production regardless of the antigen used for stimulation (Ov27 and OvD5B) or the isotype produced (IgE and IgG4) (from P < 0.05 to P < 0.001). Anti-IL-6 and anti-TNF-α antibodies synergized in all individual cultures. In contrast, addition of neutralizing
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A. Ov27-specific IgE

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B. OvD5B-specific IgE

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C. Ov27-specific IgG4

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D. OvD5B-specific IgG4

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Fig. 2. The requirement for IL-4 in B cell production of antigen-specific IgG4/IgE in response to filarial antigens and anti-CD40. OD ratios ± SEM of eight individual experiments (three for IL-13 only) are shown in response to antigens, anti-CD40 and various cytokines. Asterisks indicate that results are also significantly different ($P < 0.05$) from those in the presence of IL-4 only.

Table 1. MBP-Ov27 and MBP/OvD5B, but not MBP alone, are responsible for the production of antigen-specific IgG4/IgE antibody production when patient B cells are cultured with anti-CD40 and IL-4

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Antigen-specific antibody production (OD x 100)</th>
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<tr>
<td></td>
<td>OvD5B-specific</td>
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<tr>
<td></td>
<td>IgG4</td>
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<tr>
<td>CD19+ syse- B cells</td>
<td></td>
</tr>
<tr>
<td>+ anti-CD40 + IL-4 + MBP</td>
<td>2.12 ± 3.73</td>
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<tr>
<td>+ anti-CD40 + IL-4 + MBP/OvD5B</td>
<td>104.75 ± 9.87a</td>
</tr>
<tr>
<td>+ anti-CD40 + IL-4 + MBP/Ov27</td>
<td>ND</td>
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Purified B cells (10⁶/ml; 500 μl) were cultured with 5 ng/ml IL-4 plus 10 ng/ml anti-CD40 and optimal concentrations of MBP alone (0.01 μg/ml) or antigen (OvD5B, 0.01 μg/ml; Ov27, 0.001 μg/ml) for 14 days. Results are expressed as means ± SE from eight individual cultures. ND, not done.

Results are significantly different from control (anti-CD40 + IL-4 + MBP) ($P < 0.05$).

anti-IL-12 antibodies did not significantly alter antigen-specific antibody production. Notably, the addition of anti-IL-2 or of anti-IL-10 antibodies had differential effects on IgG4 and IgE production. For OvD5B, antigen-induced IgE production was inhibited by anti-IL-2 or anti-IL-10 antibodies (Fig. 3B), whereas OvD5B-induced IgG4 production was unaffected (Fig. 3A). For Ov27, a similar up-regulation for the IgG4 antibody was seen, but no effect was noted for Ov27-specific IgE (Fig. 3C and D).

To identify molecules possibly playing a role in the induction...
A. Ov27-specific IgE
- anti-IL-6
- anti-TNF-α
- anti-[TNF-α/IL-6]
- anti-IL-13
- anti-IL-2
- anti-IL-10
- anti-IL-12

B. OvD5B-specific IgE
- anti-IL-6
- anti-TNF-α
- anti-[TNF-α/IL-6]
- anti-IL-13
- anti-IL-2
- anti-IL-10
- anti-IL-12

C. Ov27-specific IgG4
- anti-IL-6
- anti-TNF-α
- anti-[TNF-α/IL-6]
- anti-IL-13
- anti-IL-2
- anti-IL-10
- anti-IL-12

D. OvD5B-specific IgG4
- anti-IL-6
- anti-TNF-α
- anti-[TNF-α/IL-6]
- anti-IL-13
- anti-IL-2
- anti-IL-10
- anti-IL-12

Fig. 3. Ov27- and OvD5B-specific IgG4/IgE antibody production are differentially regulated by a range of neutralizing anticytokine antibodies in vitro. CD19+ B cells from seven filaria-infected patients were cultured with either of the selected antigens and neutralizing antibodies to IL-6, TNF-α, IL-13, IL-2, IL-10 and IL-12 or relevant controls. Data are expressed as the mean percentage of controls ± SEM. Asterisks represent statistical differences at the following levels: *P < 0.05; **P < 0.01; ***P < 0.001.

Discussion
Although helminth parasites induce characteristic B cell responses (i.e. IgG4 and IgE antibody production), the nature of the interaction between the antigens themselves and the B cells that ultimately differentiate and produce these antibodies have not been well-defined. To delineate among the major factors—presumably cytokines—regulating antigen-specific IgG4/IgE production, we stimulated purified CD19+ sy− se− B cells obtained from filaria-infected individuals with either of the two recombinant filarial proteins (Ov27 or OvD5B) in the presence of anti-CD40 mAb and IL-4 as surrogates for T cell factors (6,7). Under these conditions, antigen-driven IgG4/IgE production by purified CD19+ sy− se− B cells required both antigen and IL-4, as the B cells were incapable of producing IgG4/IgE-specific antibodies in the absence of either antigen or IL-4. Additionally, Ov27- and OvD5B-specific IgG4/IgE antibodies were regulated by certain additional signals provided by recombinant cytokines (IL-13 or TNF-α), by neutralizing antibodies to cytokines (anti-IL-2, anti-IL-6, anti-IL-10 or anti-TNF-α), or by other factors such as anti-CD21, anti-CD23 or sCD23.

In the presence of IL-4 or IL-13, human PBMC can secrete IgG (IgG1, IgG3, IgG4, but not IgG2) (8), IgM and IgE (9-
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A. Ov27-specific IgE

B. OvD5B-specific IgE

C. Ov27-specific IgG4

D. OvD5B-specific IgG4

Fig. 4. Ov27- and OvD5B-specific IgG4/IgE antibody production in vitro are differentially regulated by antibodies to the B cell surface molecules CD23 and CD21. CD19+ B cells from eight filaria-infected patients were cultured with either of the selected antigens and antibodies to CD23 or CD21 (or mouse control antibodies). Data are expressed as the percentage of the controls ± SEM. Asterisks represent significant differences from controls: *P < 0.05; **P < 0.01. (A) Ov27-specific IgE antibody levels; (B) OvD5B-specific IgE levels; (C) Ov27-specific IgG4 levels; (D) OvD5B-specific IgG4 levels.

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11); this IL-4-induced Ig secretion is down-regulated by a number of other cytokines including IFN-γ, IFN-α, transforming growth factor-α, IL-10 (12), IL-8 (13) and IL-12 (14). In contrast, IL-5, IL-6, TNF-α, erythropoietin, IL-3 (12), IL-9 (15) and possibly IL-2 (16) all have been shown to synergize with IL-4 to augment immunoglobulin production. It has also been shown that, unlike IL-8 and erythropoietin, molecules that promote the expansion of a committed pool of se+B lymphocytes for the production of IgE (13,17), IL-4 (and IL-13) induce switching primarily from μ to ε (18).

We took advantage of the CD40/IL-4 culture system in which non-committal B cells can be stimulated by IL-4 (and IL-13) to produce IgG4/IgE antibodies (19-21) to understand the role the B cells themselves play in isotype selection by targeting those cytokines that can be secreted by activated B lymphocytes, including IL-1, IL-6, IL-10, IL-12, TNF-α, TNF-β (22-26), and possibly IL-13 (27) and IL-2 (28-30).

Using neutralizing anti-IL-6, anti-TNF-α and anti-IL-13 antibodies, specific antibody production was inhibited regardless of isotype, suggesting that IL-6, IL-13 and TNF-α were secreted upon B cell activation with antigen. As it has been demonstrated that IgG4/IgE antibodies produced in vitro by atopic patient B cells could be down-regulated by IL-8 acting through TNF-α and IL-6 receptors (31), and that the p55 chain of the TNF receptor has also been shown to be critically involved in CD40-induced, IL-4/IL-13-dependent IgE production (20), the present study extends these findings to include B cell responses driven by specific antigen.

Second, neutralizing anti-IL-12 antibodies did not appear to alter antigen-specific antibody production, a finding that suggests either that significant levels of IL-12 are not secreted by B cells under IL-4- and anti-CD40-dependent Ov27/OvD5B activation or that the effect of IL-12 was overcome by the IL-4 that was used in this study (32). As IL-12 can down-regulate Ov27- and OvD5B-driven IgG4/IgE antibody production by PBMC (4), and although IL-12 can act directly on B cells to regulate γ/ε switching (33), the majority of its effect is exerted through IFN-γ (32), a cytokine not present under the conditions used here.

Interestingly, both anti-IL-2 and anti-IL-10 antibodies up-regulated production of Ov27- and OvD5B-specific IgG4 antibodies but had either no effect (for Ov27) or a down-modulatory one (for OvD5B) on IgE production. This suggests that IL-2 and/or IL-10 may counteract IL-4-mediated switching
from μ to γ4 but not γE, a finding corroborated by studies in polyclonally stimulated systems (12,16,34). Although thought to be antagonists for the production of IgE, IL-4 and IL-10 strongly synergize to down-regulate production of IFN-γ in antigen-driven systems (35). Altogether, these data suggest that endogenous B cell cytokines may play a role in the isotype selection of a specific antibody produced in response to parasite antigens/allergens.

Anti-CD21 (clone BU-33) (36) and anti-CD23 antibodies very clearly showed differential effects on antigen-induced IgG4- and IgE-specific antibody production (Fig. 4). Although it has previously been shown that anti-CD23 antibodies could prevent polyclonally activated B cells from secreting IgE in the presence of IL-4 (37), its role on the human IgG subclasses has not been well studied. Although it has been hypothesized that CD23 acts as a negative feedback component of IgE regulation in helminth (Nippostrongylus brasiliensis)-infected mice (38), it has also been shown that administration of an anti-IgE antibody inhibits CD23 expression and IgE production in high-IgE producer mice (39). Altogether, the data presented here strongly suggest a role for endogenous cytokines in terminal differentiation of B cells activated by antigens, in that T cells were replaced by the engagement of CD40 and the production of IgG4/IgE was initially directed by IL-4. Our data confirm a role for IL-6 in mediating B cell responses to an antigen challenge (40) that parallels findings achieved in the CD40 culture system (26,41,42) by other groups. These data also suggest a role for IL-2 and IL-10 in the differential regulation of IgG4 versus IgE antibody production, although the level at which they operate will need to be defined at the molecular level. We also hypothesize that there is a regulatory role for FceR1a/CD23 in switching either from μ to γ4 or μ to γ4 to ε in response to certain filarial antigens (and possibly allergens). Of note, several of these cytokines/factors shown to have a co-regulatory role in Ov27- and OvD5B-driven IgG4/IgE antibody production may also act at the level of antigen presentation, as has been seen for IL-4, IL-13 (43), IL-10 (44,45) and CD23 (46). Along with previous reports (47-49), these data suggest not only that antigen-specific IgG4/IgE antibodies can be induced in vitro but also that certain epitopes on filarial proteins appear to differentially regulate overall Tc1-mediated IgG4/IgE responses. Whether this is because the naive B cell repertoire is ligand selected in infected individuals or because IgM low-affinity germline antibodies have been switched remains to be determined; what is clear, however, is that certain endogenously produced molecules from B cells such as IL-2, IL-10, CD23 and CD21 play a significant role in the production of particular isotypes of antigen-specific antibodies.

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Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>MBP</td>
<td>maltose-binding fusion protein</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
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<tr>
<td>sε</td>
<td>surface ε positive (B cell)</td>
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<tr>
<td>se</td>
<td>surface ε negative (B cell)</td>
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<tr>
<td>sγ</td>
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<tr>
<td>sγε</td>
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<td>TNF</td>
<td>tumor necrosis factor</td>
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References
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