

Original article

The complement component C3 plays a critical role in both T_H1 and T_H2 responses to antigen

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Background: Complement component C3 is synthesized by keratinocytes and is activated after skin injury. C3 is also synthesized by peritoneal macrophages, which are activated by the adjuvant alum.

Objective: We sought to investigate the role of C3 in inciting allergic skin inflammation and systemic immune responses after epicutaneous sensitization or intraperitoneal sensitization with antigen.

Methods: C3-deficient (C3^{-/-}) mice and wild-type (WT) control animals were subjected to epicutaneous sensitization with the antigen ovalbumin (OVA) on shaved and tape-stripped skin or intraperitoneal immunization with OVA in alum.

Results: Skin infiltration by eosinophils and expression of mRNA encoding the T_H2 cytokines IL-4 and IL-5 in OVA-sensitized skin sites was impaired in C3^{-/-} mice. Splenocytes from epicutaneously sensitized C3^{-/-} mice secreted less IL-4, IL-5, IL-13, and IFN- γ in response to OVA stimulation than splenocytes from WT control animals. The defect in cytokine secretion by splenocytes was also observed after intraperitoneal immunization of C3^{-/-} mice. C3^{-/-} mice had impaired IgG1, IgG2a, and IgE antibody responses after both epicutaneous and intraperitoneal immunization. The defect in cytokine secretion of C3^{-/-} mice was not due to defective proliferation to antigen, was not observed after anti-CD3 stimulation, and was corrected by the addition of purified C3 protein.

Conclusion: These results suggest that C3 plays an important role in both the T_H1 and T_H2 response to antigen *in vivo*.

Clinical implications: The complement pathway might be a potential target in the therapy of allergic diseases. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Atopic dermatitis, complement, mouse, T_H1, T_H2

The complement cascade is an important part of the innate immune system and consists of proenzymes that

Abbreviations used

APC: Antigen-presenting cell
SEA: Schistosomal egg antigen
WT: Wild-type

become activated sequentially. The major biologic consequences of complement activation are opsonization, activation of phagocytes, and lysis of target cells. Complement is activated by 3 different pathways: classical, lectin, and alternative.¹ These pathways converge on a common reaction that activates the third component of the complement system, C3. Activation of C3, the pivotal molecule in this cascade, releases several biologically active peptides from the parent molecule.

In a first step, C3 is cleaved to C3a and C3b. Subsequently, C3b is cleaved to iC3b and C3dg. Each of these peptides acts as a ligand for specific receptors to mediate classical C3 dependent functions, such as opsonization, leukocyte chemotaxis, and smooth muscle cell contraction. Expression of C3 receptors on antigen-presenting cells (APCs), T cells, and B cells suggests that C3 and its breakdown products play an important role in humoral and cell-mediated immune responses. C3d bound to its receptor on B lymphocytes lowers the threshold for B-cell activation,² whereas the effect of C3a on tonsil-derived B cells is the reduction of polyclonal antibody and cytokine synthesis.³

The effects of complement components on T-cell responses are diverse.⁴ C1q-opsonized immune complexes cause activation of T cells.⁵ C3a favors T_H1 differentiation by upregulating IL-12 synthesis in APCs.⁶ In a murine model of influenza, C3-deficient mice display diminished ability to develop cytotoxic T cells and clear the virus.⁷ C5a is chemotactic for T cells⁸ and might play a key role in delayed-type hypersensitivity⁹ and in the optimal generation of antiviral CD8⁺ T-cell responses.¹⁰

An important result of T-cell activation is functional polarization into T_H1 or T_H2 phenotypes. After antigen stimulation, T_H cells can develop into T_H1 cells that secrete IFN- γ or T_H2 cells that secrete IL-4, IL-5, and IL-13.¹¹ It has been shown that C3 is important in the generation of T_H2 effector functions in a murine model of pulmonary allergy by using a mixture of *Aspergillus fumigatus* extract and ovalbumin (OVA) and of parasitic

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infection by using schistosomal egg antigen (SEA).^{12,13} The role of C3 in T_H cell differentiation in response to protein antigen has not been thoroughly investigated. This prompted us to evaluate the role of C3 in T_H cell differentiation in 2 models of allergic sensitization: epicutaneous sensitization with antigen and intraperitoneal immunization with antigen in alum. Our results show that C3 plays an important role in both T_H1 and T_H2 responses to protein antigen.

METHODS

Mice and sensitization

C3^{-/-} mice were on a 129×C57BL/6 background¹⁴ or had been bred on the C57BL/6 background for more than 8 generations. 129×C57BL/6 and C57BL/6 wild-type (WT) mice were obtained from the Jackson Laboratory (Bar Harbor, Me). All mice were kept in a pathogen-free environment. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Children's Hospital.

Epicutaneous and intraperitoneal sensitization

Epicutaneous sensitization of 4- to 6-week-old female mice was performed as described previously (see [supplemental text](#) in the Online Repository at www.jacionline.org).¹⁵

Histologic analysis

Specimens were fixed in 10% buffered formalin and embedded in paraffin. Four-micrometer sections were stained with hematoxylin and eosin. Individual cell types were counted blind in 15 to 20 high-power fields at 1000× magnification.

Analysis of eosinophils in peripheral blood

Blood was collected after the third sensitization. The eosinophil count was determined by counting heparinized blood in a hemocytometer after staining with Discombe's fluid.

Competitive RT-PCR evaluation of cytokine mRNA in skin

This was performed as described previously (see [supplemental text](#) in the Online Repository at www.jacionline.org).¹⁶

Antibody determinations and cytokine synthesis

Antibody levels and cytokine production by cultured cells were determined as previously described (see [supplemental text](#) in the Online Repository at www.jacionline.org).⁶

Effects of C3 on cytokine production *in vitro*

Splenocytes from intraperitoneally immunized mice were cultured in 24-well plates in the presence of OVA (50 µg/mL). Purified human C3 protein (Calbiochem-Novabiochem International Inc, San Diego, Calif) was added to the wells at the beginning of the culture. After 96 hours of culture, cytokines secreted into the supernatants were analyzed by means of ELISA, as described above.

Proliferation

Splenocytes were resuspended in RPMI 1640 supplemented with heat-inactivated FCS (10%), L-glutamine (2 mM), sodium pyruvate (1 mM), 2-mercaptoethanol (5 mM), penicillin (100 U/mL), and streptomycin (100 µg/mL). Cells (4 × 10⁵) in 200 µL were plated in

flat-bottom microtiter plates and cultured in triplicate at 37°C in 5% CO₂ in the presence of a range of OVA concentrations (10-500 µg/mL) or in plates coated with different concentrations of anti-CD3ε mAb. After 3 days, cultures were pulsed for 16 hours with tritiated thymidine, cells were harvested, and radioactivity was measured in a liquid scintillation β-counter.

Statistical analysis

The Student *t* test was used to compare the differences between groups.

RESULTS

C3^{-/-} mice have impaired eosinophil infiltration and T_H2 cytokine expression in OVA-sensitized skin sites

We had previously shown that epicutaneous sensitization results in eosinophil infiltration and a predominantly T_H2 response in the skin, with increased local expression of mRNA for the T_H2 cytokines IL-4 and IL-5. [Fig 1, A](#), shows that OVA sensitization caused a significant increase in the number of eosinophils in the skin of control 129×C57BL/6 mice, which is consistent with previous observations.¹⁵ Skin eosinophil infiltration was significantly less in OVA-sensitized skin sites of C3^{-/-} mice. Decreased skin eosinophilia could be due to decreased eosinophil mobilization from the bone marrow and/or decreased eosinophil recruitment, which was previously shown to depend on IL-4 and IL-5.¹⁷ [Fig 1, B](#), shows that epicutaneous sensitization with OVA caused a significant increase in the number of circulating eosinophils in 129×C57BL/6 WT control mice. In contrast, it caused only a modest and statistically insignificant increase in circulating eosinophils in C3^{-/-} mice.

As previously reported,¹⁵ mRNA expression of IL-4 and IL-5 significantly increased in OVA-sensitized skin sites of 129×C57BL/6 WT control mice compared with saline-sensitized sites ([Fig 1, C](#)). In contrast, there was no increase in IL-4 and IL-5 mRNA expression in OVA-sensitized skin sites of C3^{-/-} mice. Also as previously reported,¹⁵ there was no detectable increase in IFN-γ mRNA expression in OVA-sensitized skin of normal mice. There was also no significant increase in IFN-γ mRNA expression in OVA-sensitized skin of C3^{-/-} mice.

Decreased secretion of T_H2 and T_H1 cytokine secretion by splenocytes of epicutaneously sensitized C3^{-/-} mice

We have previously shown that splenocytes from mice epicutaneously sensitized with OVA secrete IL-4, IL-5, IL-13, and IFN-γ after OVA stimulation *in vitro*.¹⁶ To investigate whether the decreased skin expression of cytokines in epicutaneously sensitized C3^{-/-} mice reflected a diminished systemic T_H response to antigen, we examined cytokine secretion by their splenocytes. [Fig 2, A](#), shows that OVA-stimulated splenocytes from epicutaneously sensitized C3^{-/-} mice secreted significantly less IL-13 and IFN-γ after OVA stimulation than splenocytes from epicutaneously sensitized WT control mice. IL-4 and IL-5

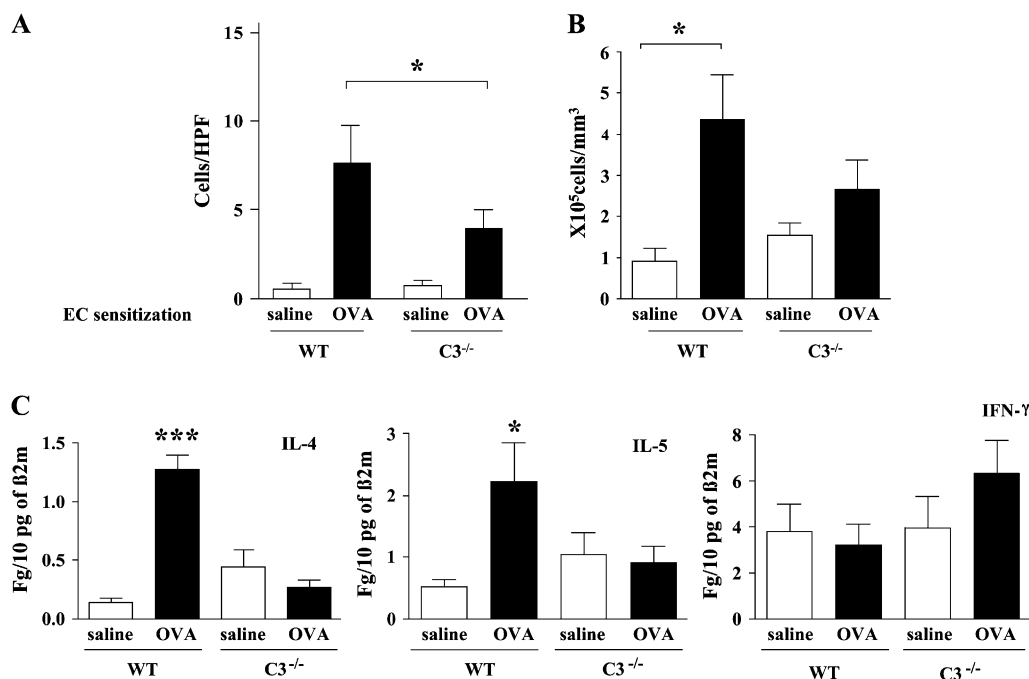


FIG 1. Skin and blood eosinophil response and skin expression of cytokines in epicutaneously (EC) sensitized C3^{-/-} mice and WT control mice. **A**, Number of infiltrating eosinophils. **B**, Blood eosinophil counts. **C**, Expression of IL-4, IL-5, and IFN-γ mRNA in OVA- and saline-sensitized skin sites of C3^{-/-} mice and WT control animals. All mice in this experiment were on the 129×C57BL/6 background. The columns and error bars represent the mean + SEM (n = 6 animals per group). **P* < .05, ****P* < .001. HPFs, High-power fields.

secretion was also diminished in C3^{-/-} mice, although the difference with control mice did not reach statistical significance. The deficiency in T_H1 and T_H2 cytokine production in response to OVA was antigen specific because splenocytes from C3^{-/-} mice secreted normal amounts of IL-4, IL-5, IL-13, and IFN-γ after stimulation with anti-CD3 (Fig 2, B). These results suggest that C3 deficiency interferes with the development of both T_H1 and T_H2 cell responses to epicutaneously introduced antigen.

Decreased OVA-specific antibody responses in epicutaneously sensitized C3^{-/-} mice

The T_H2 cytokines IL-4 and IL-13 play a critical role in IgG1 and IgE class switching, whereas the T_H1 cytokine IFN-γ plays a critical role in IgG2a class switching. C3^{-/-} mice had significantly impaired IgG1, IgE, and IgG2a antibody responses to OVA after epicutaneous immunization (Fig 3, A). Furthermore, serum levels of IgG1 and IgG2a were significantly lower in C3^{-/-} mice than those in WT control mice, whereas IgE levels were similar (Fig 3, B). These results suggest that C3 plays an important role in T_H1- and T_H2-driven isotype switching.

C3^{-/-} mice also have diminished T_H2 and T_H1 responses to antigen after intraperitoneal sensitization

C3 is synthesized by peritoneal macrophages and is activated by alum adjuvant.¹⁸⁻²² To investigate whether the impaired T_H response to antigen in C3^{-/-} mice is

restricted to the epicutaneous route of immunization, we examined the response of C3^{-/-} mice to intraperitoneal immunization with OVA in alum. Fig E1, A (available in the Online Repository at www.jacionline.org), shows that splenocytes from intraperitoneally immunized C3^{-/-} mice secreted significantly less T_H2 cytokines (ie, IL-4, IL-5, and IL-13), as well as significantly less IFN-γ, than splenocytes from WT control animals after *in vitro* stimulation with OVA.

Fig E1, B, shows that, as is the case with epicutaneous immunization, C3^{-/-} mice had significantly impaired antigen-specific IgG1, IgE, and IgG2a responses after intraperitoneal OVA immunization. This further supports the notion that C3 plays an important role in T_H1- and T_H2-driven isotype switching.

Intraperitoneally immunized C3^{-/-} mice have impaired T_H1 and T_H2 cytokine production but normal proliferation in response to a wide range of antigen concentrations

To examine the dose-response dependency of impaired T_H1 and T_H2 cytokine production in C3^{-/-} mice, we performed intraperitoneal immunization and examined cytokine production in response to an OVA concentration range of 10 to 500 μg/mL. C3^{-/-} mice on the C57BL/6 background were used because at the time we carried out this experiment, C3^{-/-} mice on the hybrid 129×C57BL/6 background were no longer available. Fig E2, A (available in the Online Repository at www.jacionline.org),

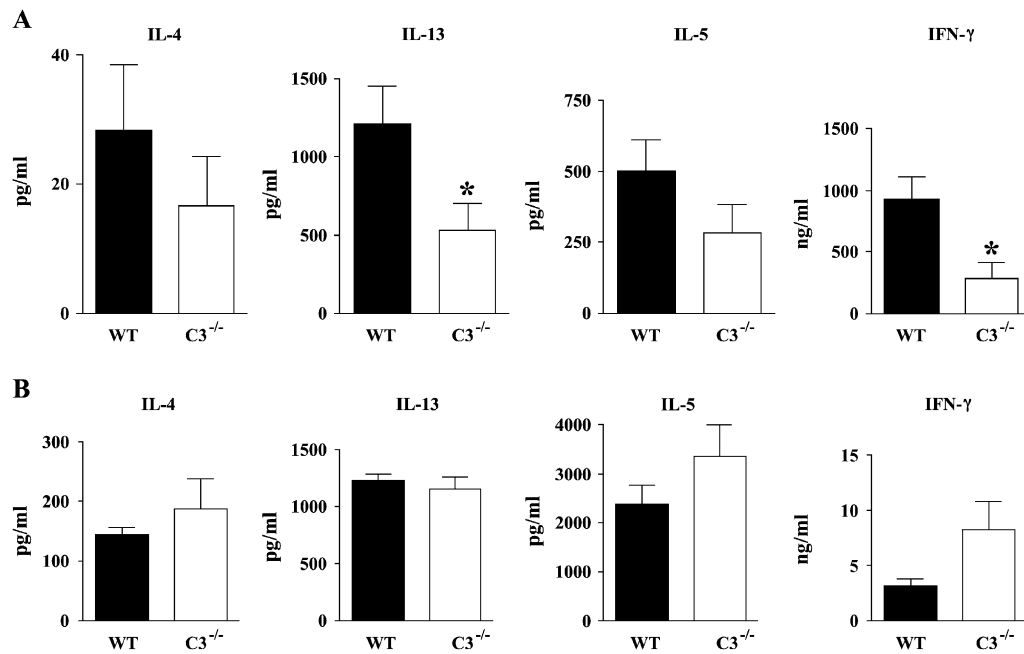


FIG 2. Cytokine levels in the supernatants of splenocyte cultures from epicutaneously sensitized C3^{-/-} mice and WT control animals. Cells were cultured in the presence of OVA (A) or anti-CD3 (B). All mice in this experiment were on the 129×C57BL/6 background. The columns and error bars represent the mean + SEM (n = 6 animals per group). *P < .05. No cytokines were detected in the supernatants of unstimulated cultures.

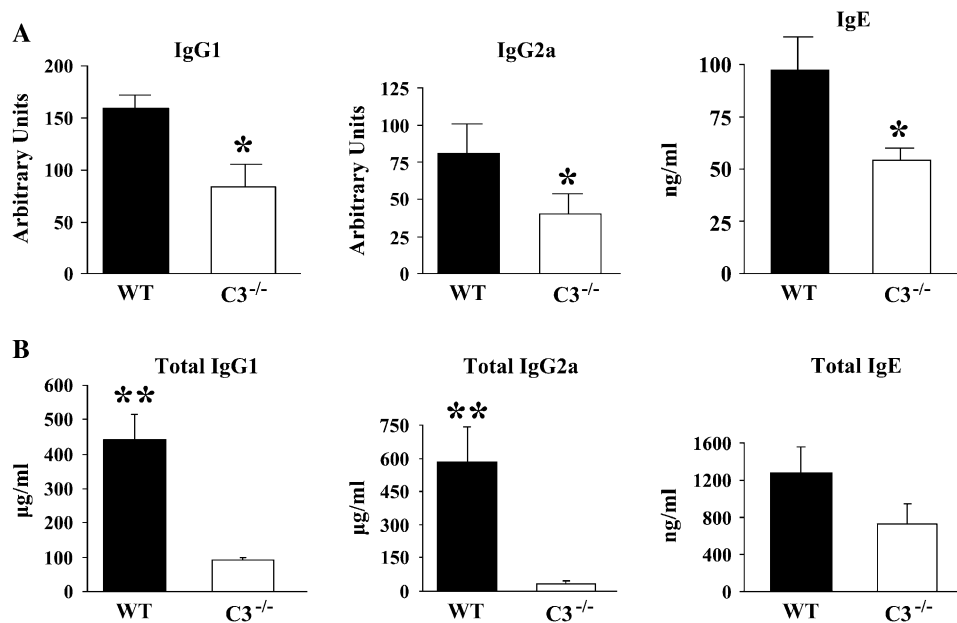


FIG 3. OVA-specific antibody levels and total serum Ig levels in epicutaneously immunized C3^{-/-} mice and WT control animals. OVA-specific IgG1, IgG2a, and IgE levels after epicutaneous immunization (A) and serum Ig levels (B) are shown. All mice in this experiment were on the 129×C57BL/6 background. The columns and error bars represent the mean + SEM (n = 6 animals per group). *P < .05, **P < .01.

org), shows that C3^{-/-} mice had impaired IL-5, IL-13, and IFN-γ production at virtually all concentrations of OVA tested. No IL-4 secretion was detectable in either WT or C3^{-/-} mice on the C57BL/6 background. As in the case of epicutaneously immunized mice, the deficiency in T_{H1} and T_{H2} cytokine production of intraperitoneally immunized

C3^{-/-} mice in response to OVA was antigen specific. Splenocytes from these mice secreted normal amounts of IL-5, IL-13, and IFN-γ after stimulation with anti-CD3 over a concentration range from 0.5 to 5 μg/mL (Fig E2, B).

The impairment of both T_{H2} and T_{H1} responses to OVA in C3^{-/-} mice raised the possibility that T-cell

sensitization to antigen might be impaired in these mice. Fig E2, C and D, (available in the Online Repository at www.jacionline.org) shows that there was no difference in the proliferation of splenocytes from $C3^{-/-}$ mice and WT control mice to OVA or anti-CD3 over the same range of concentrations used to test for cytokine production.

***In vitro* addition of purified C3 protein corrects the defect in cytokine production by splenocytes from intraperitoneally immunized $C3^{-/-}$ mice**

We examined the capacity of purified C3 to correct the defect in cytokine production by $C3^{-/-}$ mice. Because mouse C3 is not readily available, we used purified human C3, which is active on mouse cells, and examined its effect on cytokine secretion by splenocytes from intraperitoneally immunized $C3^{-/-}$ mice and WT control mice on the C57BL/6 background. Fig 4 shows that addition of huC3 corrects the defect in IL-13, IL-5, and IFN- γ secretion by splenocytes of $C3^{-/-}$ mice but has little effect on the secretion of these cytokines by splenocytes from WT control animals. HuC3 by itself had induced no detectable cytokine secretion by splenocytes (data not shown).

DISCUSSION

The present results demonstrate that production of both T_H1 and T_H2 cytokines in response to 2 independent routes of exposure, immunization with a soluble antigen and allergic skin inflammation induced by epicutaneous sensitization, were impaired in $C3^{-/-}$ mice. Furthermore, T_H1 - and T_H2 -driven antibody responses were also impaired in $C3^{-/-}$ mice. These results suggest that C3 plays a critical role in the development of T_H cells and allergic sensitization to protein antigens.

We initially explored the role of C3 in a mouse model of allergic skin inflammation induced by epicutaneous sensitization with OVA because C3 is expressed in keratinocytes and is activated after mechanical injury.²³⁻²⁵ $C3^{-/-}$ mice had diminished eosinophil infiltration and decreased expression of the T_H2 cytokines IL-4 and IL-5 in OVA-sensitized skin sites (Fig 1, A and C). The diminished eosinophil infiltration might be due to several factors that include decreased eosinophil mobilization from the bone marrow, decreased survival, and decreased recruitment to the skin. In contrast to WT mice, $C3^{-/-}$ mice failed to have significant blood eosinophilia after epicutaneous sensitization (Fig 1, B). The T_H2 cytokine IL-5 is important for eosinophil maturation and mobilization from bone marrow,²⁶ whereas both IL-5 and IL-13 are important for their survival.^{26,27} Decreased systemic IL-5 and IL-13 production, as suggested by decreased production of these cytokines by splenocytes from epicutaneously immunized $C3^{-/-}$ mice (Fig 2, A), might have contributed to decreased blood eosinophilia in these mice. Recruitment of eosinophils to the skin in our model is dependent on IL-4, which promotes eotaxin expression in skin fibroblasts,²⁸ and on IL-5, which promotes eosinophil

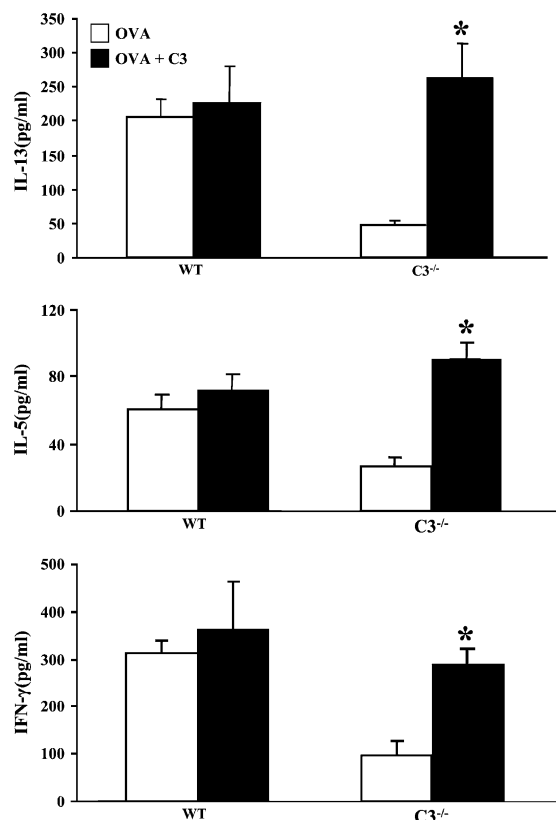


FIG 4. Effect of C3 on cytokine production after OVA stimulation by splenocytes from intraperitoneally immunized mice. Splenocytes from $C3^{-/-}$ mice and WT control animals all on the C57BL/6 background were stimulated with OVA (50 μ g/mL) in the presence or absence of C3 (20 μ g/mL), and supernatants at 96 hours were assayed for IL-5, IL-13, and IFN- γ . The columns and error bars represent the mean $\pm$ SEM (n = 5 animals per group). *P < .05.

trafficking and survival.²⁶ Decreased expression of IL-4 and IL-5 in OVA-sensitized skin sites of $C3^{-/-}$ mice might have contributed to the decreased skin eosinophil infiltration in these mice. Finally, the anaphylatoxins C3a and C5a are chemoattractants for eosinophils.^{29,30} Failure to generate C3a and impaired ability to activate C5 and generate C5a might have also contributed to decreased skin eosinophil infiltration in $C3^{-/-}$ mice.

Although our epicutaneous sensitization model results in a predominant T_H2 response in the skin with little or no up-regulation of expression of the T_H1 cytokine IFN- γ in OVA-sensitized skin sites, splenocytes from epicutaneously sensitized mice secrete IFN- γ in response to OVA stimulation.¹⁶ This T_H1 response was impaired in $C3^{-/-}$ mice. Thus these mice have a general impairment in both T_H1 and T_H2 response to epicutaneously introduced antigen. This was not the result of an intrinsic inability of their splenocytes to secrete T_H1 and T_H2 cytokines because they secreted these cytokines normally in response to anti-CD3 stimulation.

The impaired ability of $C3^{-/-}$ mice to mount systemic T_H1 and T_H2 responses to antigen was not specific to the epicutaneous route of immunization. $C3^{-/-}$ mice had impaired T_H1 and T_H2 responses to intraperitoneal

immunization with OVA. This was demonstrated by significantly decreased production of IL-4, IL-5, IL-13, and IFN- γ in intraperitoneally immunized C3^{-/-} mice on the 129 $\times$ C57BL/6 background (Fig E1, A). Significantly decreased IL-13 and IFN- γ production by splenocytes of C3^{-/-} mice paralleled the findings in epicutaneously immunized C3^{-/-} mice (Fig 2, A). IL-4 and IL-5 production by splenocytes of epicutaneously immunized C3^{-/-} mice were decreased but did not reach the level of statistical significance achieved in intraperitoneally immunized mice on the same 129 $\times$ C57BL/6 background. It is possible that C3 might play a less important role in regulating systemic IL-4 and IL-5 production than IL-13 and IFN- γ production.

Decreased production of T_H1 and T_H2 cytokines was further confirmed by using C3^{-/-} mice on the C57BL/6 background. These mice secreted significantly less IL-5, IL-13, and IFN- γ than WT control animals over a wide range of concentrations of OVA (Fig E2, A). IL-4 secretion was detected neither in WT nor in C3^{-/-} mice on the C57BL/6 background. Decreased cytokine production in intraperitoneally immunized C3^{-/-} mice was antigen specific because secretion of the same cytokines by splenocytes in response to a range of anti-CD3 concentrations was comparable in C3^{-/-} mice and WT control animals (Fig E2, B). Decreased cytokine production in response to OVA was not simply a result of decreased cell viability because proliferation of splenocytes to a range of OVA concentrations was robust and comparable in C3^{-/-} mice and WT control mice when tested at 72 hours (Fig E1, C), as well as at 48 and 96 hours, the time points when supernatants were collected for measurement of cytokine levels (data not shown).

C3^{-/-} mice had both decreased total serum IgG1 and IgG2a responses and an impaired serum antibody response of the T_H2-driven isotypes IgG1 and IgE and of the T_H1-driven isotype IgG2a in response to epicutaneous and intraperitoneal immunization with OVA (Figs 3, A, and E1, B). This decreased IgG1 response was observed in both the hybrid 129 $\times$ C57BL/6 background (Fig 3, B) and the C57BL/6 background (data not shown). Decreased serum IgG1 and IgG2a responses were noted in the initial report on C3^{-/-} mice¹⁴ but were not statistically significant. Taken together, these findings are consistent with decreased T_H1 and T_H2 cytokine production in response to antigen. The decreased IgG1 and IgG2a antibody response of C3^{-/-} mice to OVA, a T cell-dependent antigen, is in agreement with the impaired IgG1 and IgG2a response of these mice to influenza virus⁷ and with their failure to mount an IgG response to the bacteriophage Φ X174.¹⁴

In contrast to our study, which shows impaired T_H1 and T_H2 cytokine responses in C3^{-/-} mice immunized with OVA, 2 studies previously reported that C3^{-/-} mice have a selective impairment in their T_H2 response to intraperitoneal immunization.^{12,13} In the first study C3^{-/-} mice were immunized with a mixture of *Aspergillus fumigatus* culture filtrate and OVA. Impairment of the T_H2 response was evidenced by impaired accumulation of

eosinophils in bronchoalveolar lavage fluid, impaired IL-4 production in antigen-challenged lungs, and impaired IgG1 and IgE antibody responses. A normal IgG2a antibody response was taken to indicate a normal T_H1 response to OVA. In the second study C3^{-/-} mice were immunized with SEA. Secretion of the T_H2 cytokines IL-5 and IL-13 in response to SEA was impaired, whereas secretion of IFN- γ was increased. It is possible that the complex antigens used in these 2 studies might have contained an adjuvant or adjuvants that masked the effect of C3 deficiency on the T_H1 response. It is unlikely that strain differences explain the different results between these 2 studies and ours. The study with SEA as immunogen used C3^{-/-} mice on the 129 $\times$ C57BL/6 background, the study with *A. fumigatus* plus OVA used C3^{-/-} mice on the C57BL/6 background, and we have replicated our findings of decreased T_H1 and T_H2 responses in C3^{-/-} mice on the same C57BL/6 background (Fig E1).

Decreased cytokine secretion by splenocytes from C3^{-/-} mice was not the result of impaired proliferation because splenocytes from intraperitoneally immunized C3^{-/-} mice proliferated normally in response to OVA *in vitro* (Fig E2, C). It was previously reported that C3 enhances antigen uptake and presentation by APCs³¹⁻³³ and enhances the ability of a B-cell line and peritoneal macrophages to elicit the proliferation of T cells isolated from lymph nodes of OVA-injected mice in response to specific antigen.³⁴ It is possible that the effect of C3 in the latter study was specific to the particular APCs used and might not be applicable to physiologic presentation of antigen in the spleen, which involves dendritic cells.

We were able to reverse the defect in T_H1 and T_H2 cytokine secretion through the addition of C3 to splenocytes from C3^{-/-} mice. This result suggests that optimal production of T_H1 and T_H2 cytokines by *in vivo* primed T cells from these mice is dependent on the presence of C3 during encounter with antigen. This is consistent with the results of another study, in which T-cell priming, as measured by the proliferation and cytolytic activity of virus-specific T cells isolated from the draining lymph nodes of mice intranasally infected with influenza virus, was found to be deficient in C3^{-/-} mice.⁷ In contrast, T cells from C3^{-/-} mice immunized with Φ X174 provide normal help when adoptively transferred with normal B cells and antigen into irradiated normal recipients.¹⁴ This has led to the conclusion that T_H cell priming is unaffected by the lack of C3. However, in that system C3 provided by APCs of the recipient might have confounded the results.

Ligation of C3 activation products to their receptors on APCs results in the secretion of a range of cytokines. Ligation of C3a to its receptors on APCs induces the secretion of IL-12, which is important for T_H1 development.⁶ In contrast, ligation of the C3 activation product iC3b to complement receptor type 3 (the iC3b receptor) on APCs downregulates IL-2 and induces IL-10,²⁴ which favors T_H2 development.

Our results with 2 models of allergic sensitization suggest that C3 is important for the development of T_H cells *in vivo* in response to soluble antigen. Although

intraperitoneal immunization might have little bearing on the acquisition of allergic sensitivity in human subjects, epicutaneous sensitization provides a reasonably physiologic model of sensitization in patients with atopic dermatitis, which in the acute phase is characterized by skin expression of T_H2 cytokines, followed in the chronic phase by additional expression of T_H1 cytokines.³⁵ In addition to its role in T_H1 and T_H2 development, as demonstrated in this study, C3 plays other roles in allergic diseases. There is also evidence that local C3a generation at the airway surface serves as a common pathway for the induction of airway hyperresponsiveness to a variety of asthma triggers that include allergens, virus infection, and irritants.^{36,37} In addition, there is a reported association of asthma with polymorphisms in C3/C3aR genes.³⁸ Taken together with our present findings, these results suggest that targeting C3 is a potential therapeutic approach to allergic diseases.

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