AOS THESIS

Nuclear Factor Kappa-B Is Enriched in Eyelid Specimens of Rosacea: Implications for Pathogenesis and Therapy

EDWARD J. WLADIS, KEVIN W. LAU, AND ALEJANDRO P. ADAM

• PURPOSE: To assess the role of nuclear factor kappa-B (NFKB) in cutaneous specimens of rosacea and unaffected tissue.

• METHODS: Immunohistochemical staining was performed for the activated, phosphorylated variant of NFKB (pNFKB) in eyelid specimens of rosacea (n = 12) and normal, healthy tissue (n = 12). The numbers of positively staining cells/40× microscopic field were counted across 5 consecutive fields. Additionally, quantitative Western blotting was carried out for pNFKB and NFKB in specimens of rosacea (n = 15) and normal controls (n = 14). Statistical comparisons were performed via a dedicated software package.

• RESULTS: The mean number of cells/40× microscopic field that stained positively for pNFKB was 18.4 (standard deviation = 15.3) for control patients and 39.3 (standard deviation = 16.9) for rosacea patients, and the difference between the 2 groups was statistically significant (*P* = .0024). On Western blotting, the mean ratios of pNFKB:NFKB for control and rosacea patients measured 0.58 (standard deviation = 0.81) and 3.11 (standard deviation = 3.53), respectively. The 2 groups were statistically significantly different (*P* = .0002).

• CONCLUSIONS: The activated form of NFKB is enriched in rosacea, indicating a role for this pathway in the pathogenesis of this disease. Interference with NFKB signaling may represent a novel therapy for rosacea as clinical agents become available. NOTE: Publication of this article is sponsored by the American Ophthalmological Society. (Am J Ophthalmol 2019;201:72–81. © 2019 Elsevier Inc. All rights reserved.)

ROSACEA IMPACTS 16 MILLION AMERICANS, AND 58%-72% of these patients develop ophthalmic manifestations of their illness.[1] Ultimately, this disorder results in Meibomian gland disease with obstruction and scarring of the glandular orifices, cosmetic deformity from the telangiectatic vessels, and an unstable tear film, ultimately leading to tearing, discomfort, keratopathy, and vision loss.[2–6] In fact, corneal erosions, ulcerations, and perforations have all been documented in the setting of rosacea.[2,5,7]

In addition to the clinical impact of rosacea on the ocular surface, rosacea results in considerable effects on the well-being of patients. Typically, this disorder impacts patients between the ages of 40 and 59 years[8] and, consequently, interferes with work-related productivity.[9,10] Additionally, rosacea has been associated with poorer psychological quality of life.[11,12] The American Academy of Dermatology reported that rosacea results in $80 million in costs related exclusively to the cost of time spent on health care.[13]

Despite widespread recognition of the severe impact of rosacea and its deleterious effects on patients, our current therapeutic armamentarium is inadequate to address the disease, and this ailment remains incurable.[14] Lifestyle modification remains the standard of care, and patients are advised to avoid potential triggers for exacerbation of their disease.[15] Nonetheless, a wide array of aggravating factors have been implicated in rosacea, including alcohol, caffeine, chocolate, warm beverages, dairy, sunlight and heat, and emotional stress.[16] Adherence to such restrictive lifestyle may prove difficult for patients that suffer from rosacea, limiting the utility of this therapeutic approach.

Alternatively, antibiotic and antimicrobial agents have been used in rosacea.[17,18] Previous investigations have demonstrated improvements in tear breakup time, ocular surface scoring, symptoms, and eyelid appearance,[19–24] although the majority of these studies have not documented improvements in visual acuity or keratitis. The American Academy of Ophthalmology recently reported that these studies should be interpreted with caution, in light of methodologic concerns that hamper their level of evidence.[25] Furthermore, intolerances and

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side effects are quite common with these medications, including serious risks of allergy, gastrointestinal distress, photosensitivity, teratogenicity, and interference with medications.

Similarly, laser and light-based therapies may be beneficial in the management of rosacea.[26,27] These initial reports have demonstrated improvements in the tear film, in visual acuity, and in subjective dry eye–related symptoms. Despite these promising initial results, these interventions attempt to decrease inflammation in a very general sense, and do not selectively target the cellular changes inherent to rosacea. Furthermore, light-based therapies may be expensive and painful, and they may require several treatment sessions to induce relief.

Finally, surgical interventions may be necessary to alleviate the ophthalmic complications of rosacea. Punctal occlusion may heal the ocular surface.[16] Meibomian gland probing may open the scarred glands to stabilize the tear film.[28,29] Recurrent chalazia should be drained, and corneal ulceration and perforation should be managed with tissue adhesives, amniotic membrane grafts, conjunctival flaps, and penetrating keratoplasties.[1]

While all of these interventions are designed to address the damage to the ocular surface that arises from rosacea, these therapies fall short of providing durable, curative relief. In essence, our therapeutic armamentarium encompasses 2 broad strategies: suppression of inflammation in a very general sense (ie, antibiotics, intense pulsed light, etc) or management of existing damage (ie, drainage of a chalazion, Meibomian gland probing, corneal rehabilitation). Consequently, our current treatments do not target the underlying cellular and molecular changes that characterize rosacea, and, as a result, are not specific and preventive in nature.

Despite these shortcomings, an emerging body of evidence has documented key biologic aberrancies that distinguish rosacea from normal tissue, and, as a pathogenetic construct of the disease evolves, highly translational differences may be exploitable for therapeutic purposes. Specifically, Wladis and associates reported elevated levels of interleukin-1β and interleukin-6, stem cell factor, monocyte chemoattractant-1, and the monokine induced by interferon gamma in cutaneous eyelid biopsies of rosacea, as compared to normal controls.[30]

Similarly, Yamasaki and associates demonstrated enrichments in a family of membrane-spanning proteins, known as Toll-like receptors (TLRs), in rosacea.[31] These receptors identify pathogen-associated molecular patterns and, upon detection of an invading entity, initiate and coordinate a nonspecific response to remove the insult. In a follow-up evaluation that was specific to the eyelids, Wladis and associates reported increased levels of TLRs and noted that the vascular aberrancies inherent to rosacea correlated with TLR expression.[32]

Nuclear factor kappa-B (NFKB) is a transcription factor that coordinates cellular responses to a variety of stresses and serves to mediate innate immunity. In its inactive state, this protein exists as an intracellular, cytoplasmic complex, and is bound to an inhibitory protein (IKB). In response to a variety of stimuli, an enzyme called IKB kinase becomes active and phosphorylates a discrete piece of the complex. Consequently, this portion dissociates from the newly activated, phosphorylated variant of NFKB (pNFKB). The phosphorylated NFKB then translocates to the nucleus, binds to key aspects of DNA, and induces the transcription of specific DNA segments.[33–35] As a result, in direct response to multiple danger signals, pNFKB modulates gene expression, cellular behavior, and phenotype.

Multiple lines of evidence suggest a putative role for NFKB in the pathogenesis of rosacea, and the known features of this protein fit well with the current pathogenetic model of the disease. Specifically, ultraviolet light is a known aggravating factor for rosacea[16] and a well-documented trigger for NFKB signaling.[36] Additionally, NFKB is directly linked to TLRs, and, upon stimulation with a known trigger, TLRs coordinate their immune responses through NFKB.[37–39] Given the established link between TLRs and rosacea, NFKB may ultimately be involved in the pathogenesis and phenotypic features of this ailment.

Wladis and associates demonstrated enrichments of 2 critical cell signals (p38 and ERK) in cutaneous specimens of rosacea.[40] These pathways act in concert with NFKB to regulate immune responses in other conditions.[41,42]

Additionally, cathelicidin LL-37 is an antimicrobial protein that potentiates the effects of rosacea.[43] Muto and associates demonstrated that injection of LL-37 into the dermis of mice yielded a rosacea-like phenotype.[44] Although the relationship between LL-37 and NFKB has not been assessed in rosacea, these peptides are strongly linked. Specifically, NFKB mediates LL-37-related neovascularization in a model of limb ischemia.[45] LL-37 upregulates NFKB to increase expression of vascular endothelial growth factor in periodontal ligament cells.[46] Finally, LL-37 induces stem cells to undergo smooth muscle differentiation in an NFKB-dependent fashion.[47]

Taken together, the overlap between features of rosacea and the cellular features of NFKB that have been established in other settings suggests a possible role for this cell signaling mechanism in the pathogenesis of this ailment. Nonetheless, previous investigations have not explored the role of NFKB in cutaneous specimens of rosacea. An evaluation of the role of this signaling pathway carries multiple potential benefits. First, implication of a particular pathway would further clarify the pathogenetic mechanisms that ultimately result in the clinical phenotype of the disease. As such, enhanced clarity regarding the cellular aberrancies that subserve the disease could ultimately give rise to a meaningful model of this disorder.

Furthermore, identification of a critical role for the NFKB pathway presents highly translational ramifications. Specifically, interference with NFKB has been proposed as a treatment option for a variety of other ailments, including cancer,[48] autoimmune disease,[49] and osteoarthritis.[50] Through careful assessment of the cellular features that distinguish normal, healthy skin from cutaneous specimens of rosacea, novel agents that target inflammation in highly specific fashions could be introduced into the therapeutic armamentarium.

We hypothesized that NFKB is intimately involved in rosacea and is thus enriched in this disorder. This study was specifically undertaken to assess the role of NFKB in rosacea, with the intent of enhancing our comprehension of the cellular physiology of the disease and of identifying a discrete target for therapeutic manipulation.

METHODS

THESE STUDIES WERE REVIEWED AND APPROVED BY THE Institutional Review Board of Albany Medical College, and the investigations adhered to the tenets of the Declaration of Helsinki.

- **PATIENT AND SPECIMEN RECRUITMENT AND ENROLLMENT:** For purposes of recruitment of tissue specimens for Western blotting, patients with rosacea who were undergoing ectropion repair via lateral tarsal strip procedures for the management of purely involutional processes were identified from the practice of a single oculoplastic surgeon (E.J.W.). The presence of rosacea was determined by a combination of classic examination features during examination with a slit lamp (ie, eyelid margin telangiectasias, blepharitis, conjunctival hyperemia, and Meibomian gland plugging) and histopathologic confirmation by a dedicated dermatopathologist. Similarly, age- and sex-matched controls were identified during the period of institutional review board approval. Informed consent was obtained for tissue harvesting.

Additionally, in order to perform immunohistochemical analysis, a retrospective review of a case log from a single surgeon (E.J.W.) was performed. A second cohort of patients who underwent ectropion repair were identified, and charts were reviewed to identify specimens from patients with and without rosacea. Paraffin-embedded specimens were retrieved from the pathology archives, and serial sections were created.

- **WESTERN BLOTTING:** During ectropion repair, cutaneous specimens of the anterior lamella of the eyelid were immediately frozen at -80 C for later use. For purposes of Western blotting analysis, each sample was thawed, weighed, and processed for protein extraction. Specifically, 300 μL ice-cold lysis buffer containing 1% Triton X-100 (Sigma-Aldrich, St. Louis, Missouri, USA) in phosphate-buffered saline (PBS) containing protease and phosphatase inhibitor cocktails (Roche, Basel, Switzerland) and 50 mM pervanadate (Sigma-Aldrich, St. Louis, Missouri, USA) was added to the sample, together with 250 mg of zirconia/silica beads (Biospec Products Int, Bartlesville, Oklahoma, USA). Then samples were homogenized through 3 cycles of 1 minute each on a Mini-Beadbeater-96 (Biospec Products Int, Bartlesville, Oklahoma, USA). Samples were centrifuged at >12 000*g* for 2 minutes at 4 C and the supernatant was cleared by a second centrifugation for 15 minutes at >12 000*g* for 2 minutes at 4 C. Lysates were aliquoted. These aliquots received 1× volume of 2× Laemmli buffer and were boiled for 5 minutes. A mix of equal parts of each lysate was run on each gel and used for normalization purposes.

Western blots were performed using 10 μL of lysate/lane and detected using antibodies against phosphorylated (Ser536) and total NFKB (Cell Signaling, Danvers, Massachusetts, USA). Horseradish peroxidase–conjugated secondary antibodies were from Jackson ImmunoResearch. Signal was detected with Clarity Western ECL Substrate (Bio-Rad, Hercules, California, USA) and a FujiFilm LAS-3000 imager. Band quantification was performed using FujiFilm MultiGauge software from raw image files according to manufacturer's instructions.

- **IMMUNOHISTOCHEMISTRY:** Paraffin-embedded sections of a separate cohort of eyelids that were obtained during ectropion repair were deparaffinized and rehydrated with sequential steps in xylene and ethanol solutions (100% → 95% → 70%). Endogenous peroxidase activity was blocked with 0.5% H_2O_2/MeOH for 10 minutes at room temperature (RT) and antigen retrieval was done for 30 minutes at 100 C in 10 mM citrate buffer, pH 6. Samples were blocked with 5% normal goat serum (Vector S-1000, Burlingame, California, USA) for 1 hour at room temperature. Primary antibodies were incubated at a dilution of 1:80 in PBS overnight at 4 C. Biotinylated anti-mouse secondary antibodies (Vector ba-9200, Burlingame, California, USA) 1:500 in PBS were incubated for 1 hour at RT. Then, samples were incubated with avidin/biotin peroxidase (Vector ABC kit Elite PK-6100) in the dark for 30 minutes at RT and signal was detected with 3,3'-diaminobenzidine (Immpact DAB, Vector SK-4105, Burlingame, California, USA) and counterstained with hematoxylin (Vector H-3404) prior to dehydration and mounting with VectaMount (Vector H-5000, Burlingame, California, USA). Positively staining cells were quantified per 1000× field.

The numbers of positively staining cells for pNFKB per 40× microscopic field were counted by 1 observer across 5 consecutive fields during routine light microscopy.

- **STATISTICAL ANALYSIS:** The total and phosphorylated variants of NFKB were quantified and were recorded in an Excel (Redland, Washington, USA) spreadsheet. Each of these numbers was then divided by the quantification value for the master mix, and a ratio of pNFKB to total NFKB was calculated for each specimen.

Similarly, the total number of cells/40× field was recorded for each specimen in an Excel spreadsheet. The means and standard deviations for each group were calculated.

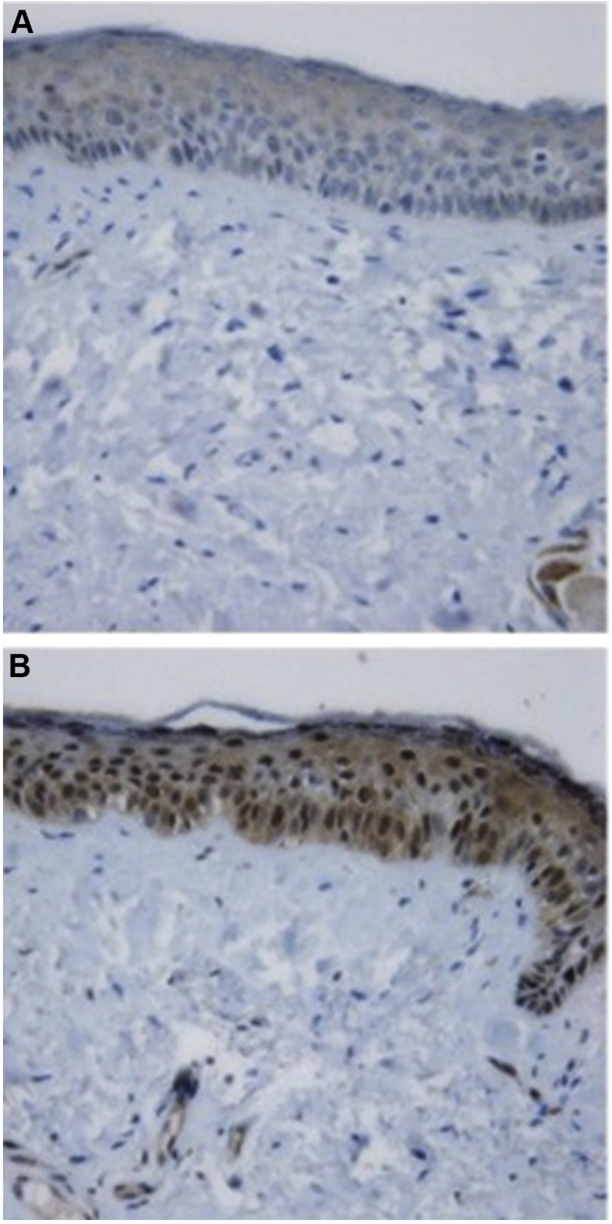

FIGURE 1. Representative 40× photomicrograph of immunohistochemical staining for phosphorylated variant of nuclear factor kappa-B (pNFKB) in a control eyelid (A) and an eyelid from a patient with rosacea (B). When present, pNFKB stains in brown.

The statistical significance of these findings was calculated using a Mann-Whitney comparison, using a dedicated statistical software package (GraphPad Prism, La Jolla, California, USA). *P* values of .05 or less were considered statistically significant.

RESULTS

- **IMMUNOHISTOCHEMISTRY:** The eyelids of 12 patients with rosacea (6 men, 6 women, mean age = 57.1 years, standard deviation = 6.9 years) were compared to 12 age- and sex-matched controls (6 men, 6 women, mean age = 56.4 years, standard deviation = 7.3 years). The difference between the ages of the 2 groups was not statistically significant.

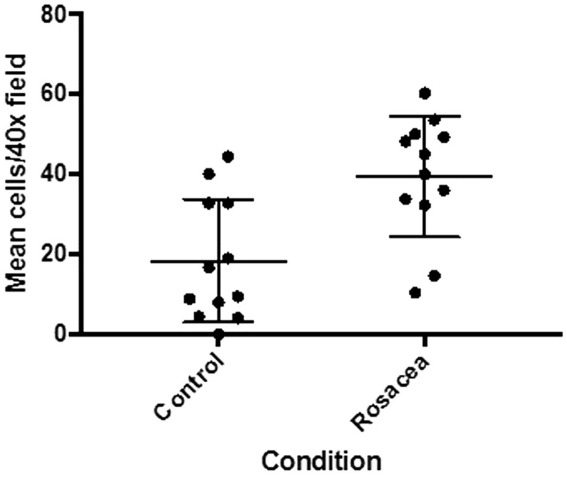

FIGURE 2. Graphical representation of the mean number of positively staining cells for phosphorylated variant of nuclear factor kappa-B in control specimens and rosacea specimens.

For control patients, the mean number of cells that stained positively for pNFKB was 18.4 cells/40× microscopic field (standard deviation = 15.3 cells/40× microscopic field). Among patients with rosacea, the mean number of positively staining cells was 39.3 cells/40× microscopic field (standard deviation = 16.9 cells/40× microscopic field). On statistical analysis, the difference between the 2 groups was statistically significant (*P* = .0024).

Figure 1 shows a representative photomicrograph of immunohistochemically staining for pNFKB in eyelids of control and rosacea patients. Figure 2 is a graphical depiction of the mean number of positively staining cells/40× microscopic field for all patients.

- **WESTERN BLOTTING:** Western blotting was performed on cutaneous eyelid samples taken from a different subset of 14 control patients (7 men, 7 women, mean age = 58.6 years, standard deviation = 4.3 years) and 15 patients with rosacea (7 men, 8 women, mean age = 57.5 years, standard deviation = 4.4 years). The difference between the ages of the 2 groups was not statistically significant.

For control patients, the mean ratio of pNFKB to total NFKB measured 0.58 (standard deviation = 0.81), whereas the mean ratio for rosacea patients was 3.11 (standard deviation = 3.53). The 2 groups were statistically significantly different (*P* = .0001).

Figure 3 shows representative Western blotting for pNFKB in rosacea and control patients. Figure 4 is a graphical representation of the ratios of pNFKB to total NFKB.

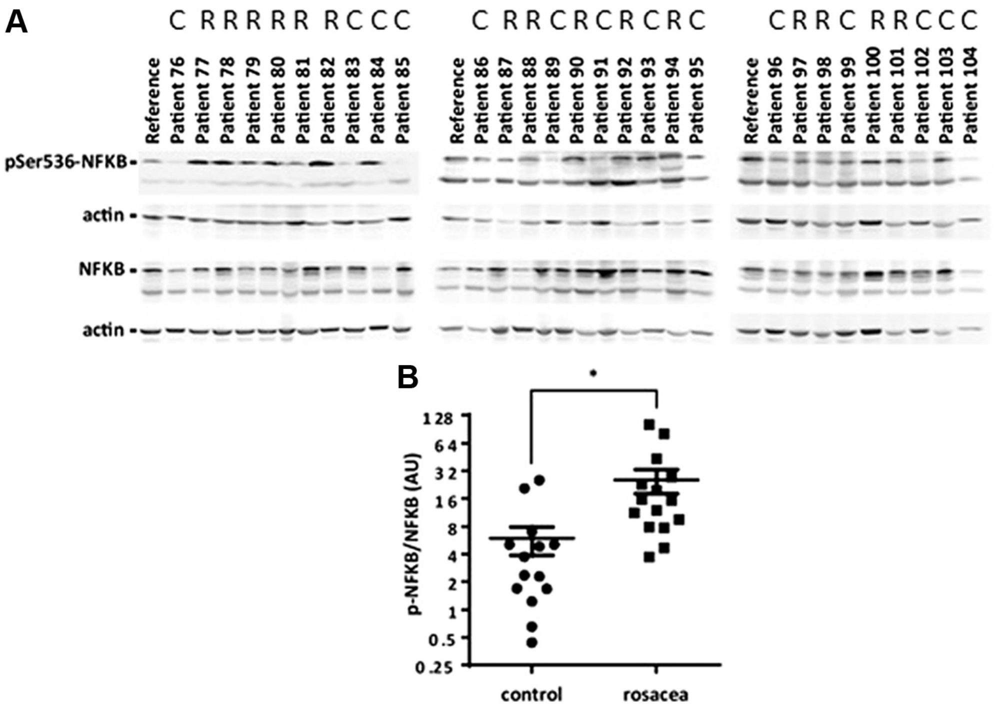

FIGURE 3. **(A) Western blot of total nuclear factor kappa-B (NFKB) and phosphorylated variant of NFKB (pNFKB) in control and rosacea specimens. C = control, R = rosacea. (B) Graphical representation of the ratio of pNFKB/NFKB.**

DISCUSSION

THESE EXPERIMENTS IMPLICATE THE NFKB SIGNALING pathway in the pathogenesis of rosacea. In essence, the activated, phosphorylated variant of this protein is enriched in this disorder, as compared to specimens of unaffected skin. The fact that both a low-throughput technique (ie, immunohistochemical analysis) and a higher-throughput modality (ie, Western blotting) yielded the same result strongly associates NFKB with the disease.

For purposes of this study, eyelid tissue was used for analysis. The results of this investigation may be highly relevant to the ocular surface changes that emerge from rosacea. Specifically, while corneal dryness and subsequent pain, photophobia, and blurred vision are hallmarks of this disorder, rosacea is fundamentally a cutaneous disease. As such, these experiments provide further insight into the pathogenesis of the skin-related aberrancies that ultimately result in rosacea-related ocular surface disease.

Nonetheless, the classification of rosacea has evolved over the past several years. In 2002, the National Rosacea Society described 4 subtypes of the disease, including erythematotelangiectatic rosacea, papulopustular rosacea, phymatous rosacea, and ocular rosacea.[51] More recently, this disorder has been formally reclassified, with the intent of focusing on symptoms and clinical findings.[52] In essence, the revised classification necessitates that at least 1 primary characteristic of rosacea (ie, facial erythema, phymatous changes, etc) be present to make the diagnosis. The ophthalmic manifestations of the disease are considered to be a secondary phenotype of the disease, and are grouped alongside papules, pustules, telangiectasias, and facial flushing. Consequently, the ocular findings inherent to rosacea are now considered to be part of an overall spectrum of the cutaneous disease, suggesting that the role of NFKB that is delineated through these experiments is not specific to the eyelid, but, instead, represents a critical feature of rosacea wherever it arises.

Enrichment in the activated form of NFKB fits well with the previously identified functions of this protein. Specifically, ultraviolet light is a known trigger for rosacea,[16] and, similarly, this stimulus increases NFKB activation.[36,53–55] Additionally, rosacea is associated with increased colonies of bacteria and mites, including bacillus and demodex.[56–61] Though the impact of these particular pathogens on NFKB activity has not been expressly studied, this signaling pathway is directly associated with immune responses to invading agents.[62,63] Moreover, the enhanced NFKB activation clearly demonstrated in rosacea in the current study lends additional credence to the concept that this disorder may represent an exuberant reaction to microbial invasion and may stem from underlying bacterial infection.

Furthermore, increased NFKB activity is consistent with the known cellular aberrancies of rosacea. Of note, several previous investigations have demonstrated enrichments of TLRs in cutaneous biopsies of rosacea, as compared to

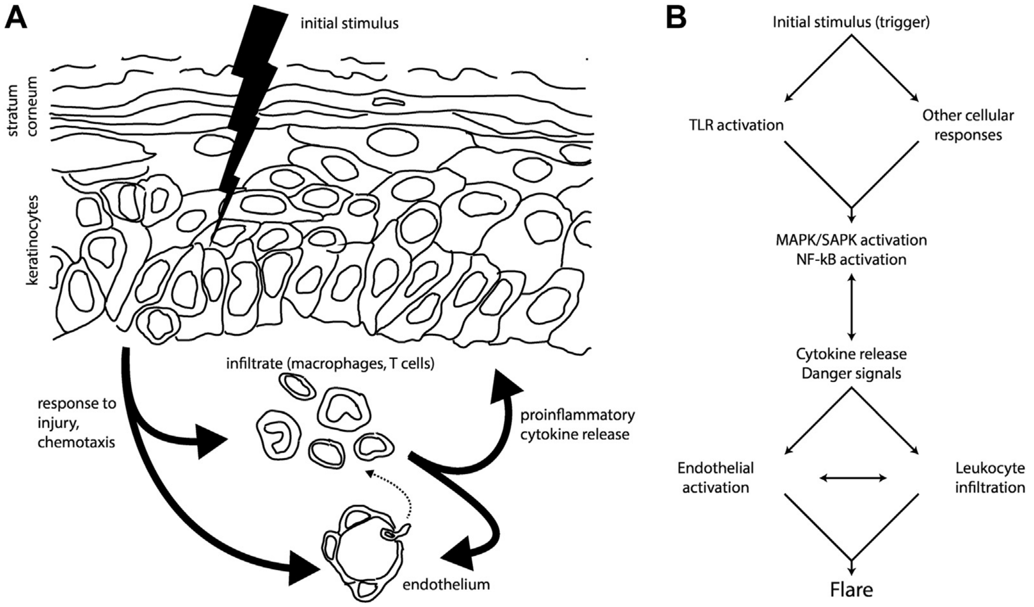

FIGURE 4. Schematic model of the exacerbated skin response in ocular rosacea. (A) An initial stimulus, often called a "trigger," that normally does not cause a significant epidermal reaction, induces a stress response in the rosacea epidermis, which is then followed by the release of a mixture of cytokines and danger signals to elicit endothelial activation and leukocyte recruitment. This creates an inflammatory response via a self-feeding cycle of cytokine release and tissue damage. (B) The triggers may act by activating Toll-like receptors and/or other cellular stress responses, leading to MAPK and SAPK activation (ie, MEK/ERK and p38) as well as nuclear factor kappa-B activation. This intracellular signal in turn promotes a transcriptional response and release of proinflammatory cytokines, chemokines, and other danger signals, which are received by Toll-like receptors and cytokine receptors in endothelial cells and leukocytes that amplify the response.

unaffected controls.[31,32] These membrane-spanning proteins provide surveillance against pathogens and, upon detection of such an invader, initiate and coordinate a nonspecific immune response.[39] Ultimately, upon stimulation of TLRs, all of the known pathways ultimately result in NFKB activation.[64]

Similarly, TLR stimulation results in the activation of a serine protease known as kallikrein-related peptidase 5,[43] which activates the antimicrobial protein cathelicidin LL-37.[65] LL-37 is associated with rosacea, and injection of LL-37 induces a murine model of the disease.[43,44] In other settings, NFKB appears to mediate the effects of LL-37,[45–47] thereby further linking this signaling pathway to the previously identified cellular changes inherent to rosacea.

In an analysis of the molecular biology of rosacea, Wladis and associates compared the concentrations of a variety of cytokines and chemokines in cutaneous biopsies of the disease and age- and sex-matched controls.[30] That study documented enrichments of interleukin (IL)-1β and interleukin-16, stem cell factor, monocyte chemoattractant protein-1 (MCP-1, CCL2), and the monokine induced by interferon gamma (MIG, CXCL2).

Notably, several of these molecules are associated with the NFKB signaling pathway in other experimental conditions. Specifically, NFKB activity was dramatically enhanced in endothelial cells after treatment with IL-1β.[66] Additionally, porcine uterine epithelial cells upregulated NFKB after they were treated with IL-1β.[67] After exposure to beta-amyloid, neural tissue expressed elevated levels of both NFKB and MCP-1.[68] Inhibition of NFKB prevents the expression of MCP-1.[69] NFKB is critical to the production of MIG expression after hepatitis B infection[70] and during osteoclast differentiation.[71]

In a separate investigation, Wladis and associates used multiple different techniques to demonstrate enrichments of intracellular signals known as p38 and ERK in cutaneous biopsies of rosacea.[40] Other experimental models have documented associations between NFKB and these signals.[41,42] Furthermore, p38 and NFKB appear to behave in a synergistic fashion to mediate inflammatory responses. Specifically, when stimulated with an infectious challenge, p38 controls transcription of NFKB in phagocytic cells.[72] Additionally, in chondrocytes, specific inhibition of p38 results in decreased NFKB expression.[73]

Interestingly, the association between ERK and NFKB is even stronger. Continuous ERK activation is required to induce IL-1β to activate NFKB, and ERK appears to act as a control mechanism for the stimulation of NFKB and for the ultimate expression of NFKB-related genes.[74] In oligodendrocytes, ERK inhibition suppressed NFKB activation after an infectious challenge.[75] In light of these associations, the heightened activity of NFKB documented in these experiments corresponds with previous research into the biology of rosacea.

More critically, the identification of a role for NFKB in the development of rosacea serves to refine our comprehension of the disease and further clarifies its cellular underpinnings. Of course, NFKB activation is a common consequence of multiple stimuli. However, several key aspects of rosacea have been delineated in other studies. Based on the disparate features associated with the cell biology of rosacea, a pathogenetic construct of the disorder can be developed. Presumably, some pathogen-associated molecular pattern stimulates the membrane-spanning TLRs. After activation, the TLRs induce an intracellular immune response, which activates p38 and ERK and culminates in NFKB phosphorylation and activation. This protein then translocates to the nucleus and induces the transcription of specific genes, ultimately leading to the production of critical effector molecules and the phenotype of rosacea. Figure 4 shows a representative figure of this model.

From a translational point of view, activation of the NFKB signaling pathway carries important therapeutic implications. In light of the multiple roles that NFKB plays in the pathogenesis of several different ailments, treatment strategies have been developed to selectively manipulate this pathway.[48–50] Given this enthusiasm for NFKB modulation, several reports have documented markedly novel approaches. For instance, conjugation of a medication that suppresses NFKB upregulation improved pancreatic transplant survival in a mouse model.[76]

NFKB inhibition has been employed in the management of other cutaneous diseases, and initial investigations suggest that this strategy may be effective as a future modality. For example, an NFKB inhibitor demonstrated in vitro efficacy against a melanoma cell line.[77] Inhibition of NFKB was associated with improvement in atopic dermatitis in a mouse model.[78] The application of an agent that blocks nuclear translocation of NFKB reduced photodamage in hairless mice.[79] While these investigations are preclinical in nature, they underscore the potential utility of suppression of this signaling pathway as a mechanism to counter a variety of skin-related ailments.

Similarly, clinical studies have indicated a role for NFKB inhibition in the therapy of cutaneous disease. The proteasome inhibitor bortezomib was used in a phase II study for the management of melanoma, although the toxicity associated with its use was significant.[80] This class of medication blocks the degradation of the IKB inhibitory protein, thereby preserving the quiescent, inactive state of NFKB. Despite these promising possibilities, much of the therapeutic complexity inherent to NFKB inhibition stems from the notion that this signaling pathway serves a variety of complex systemic functions.[81] Nonetheless, given the cutaneous nature of rosacea, topical administration of a medication might alleviate some of these concerns, by avoiding the toxicity and side effects associated with systemic absorption.

This study documents enrichments of pNFKB in cutaneous specimens of rosacea, and thus assigns a mechanism of transcriptional control to this signaling pathway in the setting of this disease. In essence, although this study does not implicate specific segments of DNA in rosacea, it defines a discrete regulatory process of transcription that is specific to the ailment. Moreover, the implication of this mechanism further clarifies the biology of rosacea. However, while this study helps to characterize the unique form of transcription in rosacea, the genetic signatures that are inherent to the disease have only recently emerged, and additional investigations could further our comprehension of the cellular aberrancies intrinsic to this ailment. Preliminary research into genetic changes in rosacea has begun to yield promising results. Chang and associates reported the results of a genome-wide association study in a large number of rosacea patients and control subjects, and noted discrete polymorphisms in the disease.[82] Aponte and associates used a similar approach in a larger cohort of patients, and defined specific loci that distinguished patients with the disease from healthy controls.[83]

These genome association studies implicated several immunoregulatory loci in rosacea, highlighting the potential role for an inflammatory reponse, and hence further implicating NFKB as a major proinflammatory transcription factor. Certainly, some of the loci that have been ascribed to rosacea have been previously associated with NFKB, and investigations from other fields may relate these gene loci with this signaling pathway. Specifically, both IRF4 and NFKB are overexpressed in rheumatoid arthritis.[84] Dysregulation of NFKB and PSMB9 is related to vitiligo.[85] NFKB and NRXN3 are both associated with bladder outlet obstruction.[86]

Of course, these investigations are preliminary in nature, and they only serve to suggest a possible association between previously implicated genetic variants and the NFKB signaling pathway. However, by assessing the transcriptome itself, one could theoretically determine the genes that are truly transcribed and thus impact phenotype in a meaningful manner. RNA sequencing could ultimately facilitate the definition of specific genetic aberrations in rosacea.[87]

Additionally, the identification of particular features of the disease could facilitate the development of animal models of rosacea that could further refine our understanding of the disease and provide a meaningful mechanism for future study. Specifically, one could upregulate each individual facet of the disease (ie, expression of TLRs, NFKB, p38, etc). Finally, selective interference with each of these pathologic aspects provides fertile ground for therapeutic manipulation, and therefore carries the promise of developing the highly selective, highly targeted treatments that patients with this currently incurable ailment truly deserve.

Despite the promise of these investigations, the results must be juxtaposed against certain inherent limitations. Specifically, this study demonstrates enrichments of activated NFKB, but it does not define its role, and future, more mechanistic approaches may be necessary to delineate the role of this protein in rosacea. The role of NFKB in other inflammatory skin conditions also merits consideration, as this factor may play a role in other diseases; in light of the role of NFKB in other systemic inflammatory diseases, the enrichments defined in the current study may not be specific, and NFKB may be influential in other cutaneous ailments. Given the sample sizes employed in this study and the relatively large standard deviations, future research that uses larger patient cohorts may confirm the robustness of these results. Similarly, while NFKB is a promising target in the management of rosacea, clinical suppression of transcription factors is a technically challenging therapy.

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