

Allergen immunotherapy now and in the future

Harold S. Nelson, M.D.

ABSTRACT

Background: Subcutaneous (SCIT) and sublingual (SLIT) immunotherapy provide effective treatment for allergic rhinitis and allergic asthma with clinical improvement following an adequate course of therapy persisting in most patients for years after treatment is discontinued. However, both require prolonged courses of therapy and many or most patients either do not begin or stop long before they have completed the prescribed course of treatment.

Methods: Based on review of the recent medical literature, the current status of SCIT and SLIT was reviewed as well as new approaches to allergy immunotherapy (AIT) that have promise to overcome the safety and inconvenience concerns of both the current approaches.

Results: New approaches to AIT include application of extracts to the skin with patches, injection into inguinal lymph nodes, alterations in the allergen molecules by chemical treatment or recombinant technology to make them less reactive with specific IgE, shifting the immune response by stimulation of toll-like receptors or suppression of Th2 responses, and finally by adjuvants such as probiotics and vitamin D.

Conclusions: Current forms of immunotherapy require years of treatment. New approaches, although differing markedly in their approach to AIT, all offer marked reduction in the required period of treatment. Hopefully, some of these new approaches will prove safe and effective and obtain approval for general use. If approved, they should make AIT more widely utilized to the benefit of the allergic population.

(Allergy Asthma Proc 37:268–272, 2016; doi: 10.2500/aap.2016.37.3966)

Subcutaneous immunotherapy (SCIT) is a long-established treatment option in the United States for allergic rhinitis, allergic asthma, and Hymenoptera venom sensitivity. Recently, sublingual immunotherapy (SLIT) using tablets of grass or ragweed extract has been approved, and, in addition, an increasing number of allergist/immunologists are treating patients sublingually with the liquid extracts that are approved only for injection use.¹ Although the use of SCIT was for a long time based only on empiric evidence, in recent decades, quality studies provided scientific evidence to support these empiric observations^{2–6} (Table 1). Clinical efficacy has been well documented for allergic rhinitis, asthma, Hymenoptera venom sensitivity and, atopic dermatitis in selected patients.² A number of double-blind, placebo-controlled studies defined effective and, in some cases, less effective doses in terms of the major allergen content.³ Several old, but well-conducted studies are reassuring that administration of multiallergen mixes is effective.³ SCIT is accepted as

modifying the immune mechanisms that underlie allergy, which results in prevention of new sensitization in patients who are monosensitized⁴ and progression to asthma in patients with only allergic rhinitis.⁵ Also, results of studies have demonstrated persisting remission in the majority of subjects for several years after completion of 3 to 5 years of treatment.⁶

Many of these same attributes have been demonstrated for SLIT (Table 2). Efficacy has been demonstrated for the same allergic conditions, although SLIT is not currently recommended for Hymenoptera venom sensitivity.⁷ Large, multidose studies have defined effective and ineffective doses for grass, ragweed, and house-dust mite tablets.⁷ SCIT has been demonstrated to have the same disease-modifying properties, including prevention of progression to multisensitization and asthma, and persistence of clinical improvement for several years after cessation of successful treatment.⁸

However, there are problems with both SCIT and SLIT that limit their use to a small percentage of those patients who might benefit from the treatment (Table 3). Safety is a concern with SCIT, and here the advantage lies with SLIT, which is much less apt to cause systemic reactions and fatalities have not been reported. Particular problems for SLIT are the lack of defined doses for the liquid extracts and lack of evidence of efficacy for mixtures of more than two allergen extracts administered simultaneously.⁹ There is only one study that examined administration sublingually of a mixture of more than two allergen extracts.⁹ In this study, the same dose of timothy extract was administered mixed either with saline so-

From the Department of Medicine, Allergy/Immunology Division, National Jewish Health, Denver, Colorado, and School of Medicine, University of Colorado, Denver, Colorado

Presented at the Eastern Allergy Conference, June 5, 2016, Palm Beach, Florida
H. Nelson is a consultant to Merck, Circassia, AstraZeneca, and Pearl Therapeutics, and is a grant recipient of Circassia

No additional external funding sources reported

Address correspondence to Harold S. Nelson, M.D., National Jewish Health, Department of Medicine, Allergy/Immunology Division, 1400 Jackson Street, Denver, CO 80206

E-mail-address: nelsonh@njhealth.org

Copyright © 2016, OceanSide Publications, Inc., U.S.A.

Table 1 The present status of subcutaneous immunotherapy

Proven efficacy in allergic rhinitis, allergic asthma, Hymenoptera venom sensitivity, and atopic dermatitis in selected patients
 Identified effective doses
 Effective in multiallergen mixes
 Demonstrated prevention of
 1. New sensitization in patients who are monosensitized
 2. Progression from rhinitis to asthma
 Established duration
 Persistence of efficacy after stopping

Table 2 The present status of SLIT

Efficacy with monotherapy for allergic rhinitis and allergic asthma atopic dermatitis in selected patients
 Appropriate dosing for SLIT tablets for grass, ragweed, and house-dust mites
 Optimal duration
 Prevention of
 1. New sensitization in individuals who are monosensitized
 2. Progression from rhinitis to asthma
 Persistent benefit after stopping
 Improved safety in comparison with SCIT

SLIT = sublingual immunotherapy; SCIT = subcutaneous immunotherapy.

Table 3 Problems with current subcutaneous and sublingual immunotherapy

Problems with current subcutaneous immunotherapy
 Safety
 Inconvenience
 1. Injections administered in physicians' offices
 2. Prolonged duration of therapy (3–5 y)
 Poor adherence
 Problems with sublingual immunotherapy
 Prolonged duration of therapy (3–5 y)
 Poorly defined doses for liquid extracts
 Lack of demonstrated efficacy for simultaneous administration of more than two unrelated extracts
 Poor adherence

lution or with nine other unrelated pollen extracts, and both were compared with placebo. After 10 months of SLIT, the timothy monotherapy showed significant production of specific immunoglobulin G4 and an increase in the threshold for both titrated nasal and cu-

Table 4 Strategies to reduce the number of injections and increase the efficacy of AIT

Change to a more efficient route of administration
 Increase the dose of allergen by reducing its allergenicity by
 1. Chemical modification (allergoid)
 2. Recombinant modification
 3. Non-IgE binding peptides
 Use of a Th1 or Treg promoting TLR ligand
 1. CpG oligonucleotides
 2. MLP
 Suppression of Th2 response
 1. Th2-cytokine antagonists
 Adjuvants
 1. Probiotics
 2. Vitamin D

AIT = allergy immunotherapy; CpG = cytosine-phosphate-guanine; IgE = immunoglobulin E; Th = T helper; Treg = regulatory T cell; TLR = Toll-like receptor; MLP = mono-phosphoryl lipid A.

taneous challenges. The same dose of timothy, administered at the same concentration but mixed with other pollen extracts, showed significant changes versus placebo only for the titrated skin-prick test and then to a markedly less degree than was seen with the monotherapy.⁹

Both SCIT and SLIT require 3–5 years to ensure persisting benefit after stopping treatment, with SCIT having the added disadvantage that the treatment must be administered in a medical facility. It is probably this requirement for prolonged treatment that accounts for many suitable subjects electing not to receive allergy immunotherapy (AIT) and for the very high rate of discontinuation before completion of a full course of treatment. In one study in the Netherlands, 23% of patients prescribed SCIT and only 7% of patients prescribed SLIT continued in treatment for 3 years.¹⁰ The relative efficacy of SCIT and SLIT is still a matter of contention, although there currently is available evidence of somewhat greater efficacy, at least in the first year, for SCIT.¹¹

ALTERNATIVE APPROACHES TO IMMUNOTHERAPY

There are a number of approaches that are being investigated to improve AIT (Table 4), which can be placed into four categories: (1) change in the route of administration, (2) modification in the allergen, (3) stimulation of innate immune responses, and (4) use of an adjuvant.

Epicutaneous and Intralymphatic Administration

Treatment can be made more effective by administering the allergen extract to tissue that has a high

density of antigen-presenting cells and can be safer by administering to a tissue with a low density of mast cells and little vascularity.¹² These conditions are met with both epicutaneous and intralymphatic allergen administration. A group in Switzerland reported the results of three studies of epicutaneous AIT.¹³ Initially, they administered grass extract by using 12 patches at 1-week intervals, with each patch remaining on for 48 hours. In later studies, they reduced the number of patches to six at weekly intervals and the duration of application to 8 hours. Application of each patch was preceded by repeated stripping of the skin with transparent tape. With this treatment, they demonstrated reduction of symptoms in two successive grass pollen seasons without any treatment before the second year. The adverse effects in the third study consisted of eczema, with 18% of the patches and 4% of subjects experiencing mild systemic symptoms.

The same group reported a large, open trial of intralymphatic AIT in which one group received conventional high-dose grass SCIT for 3 years, receiving a total of >4 million standardized quality of timothy extract, whereas the other group received three intralymphatic injections each of only 1000 standardized quality of timothy at 4-week intervals.¹⁴ The intralymphatic injections were well tolerated in regard to discomfort and induced fewer systemic reactions than the conventional SCIT. After 4 months, the nasal challenge threshold had increased with the intralymphatic AIT but not with SCIT, and, after 3 years, there was no difference between the two groups in nasal allergen challenge or in any upper or lower respiratory symptoms. Subsequently, four smaller but blinded studies have been reported. Three studies administered the intralymphatic injections at 4-week intervals and reported positive results.^{15–17} One group of investigators administered the injections at 2-week intervals and reported no clinical improvement.¹⁸

At present, both of these approaches seem to have promise. The epicutaneous approach requires only six weekly patches, five of which are applied by the patient at home, whereas intralymphatic immunotherapy involves three lymph node injections at 4-week intervals; both demonstrated persisting benefit for 1 and 3 years, respectively.

Modification of the Allergen by Chemical Treatment or Recombinant Technology

Chemical modification of allergens by exposure to formaldehyde or glutaraldehyde to convert them to allergoids is not a new approach. The treatment alters the three-dimensional structure of the molecule, which reduces the ability to react with specific immunoglobulin E but retains the T-lymphocyte epitopes. A number of allergoids have been marketed in Europe but

often without published documentation of efficacy. In fact, an analysis of four of the European allergoids indicated that both the reactivity with immunoglobulin E and the ability to stimulate T lymphocytes were similarly reduced by the process.¹⁹ However, a recent study of a grass allergoid seemed to overcome these concerns.²⁰ In a double-blind, placebo-controlled study of 121 patients, the up-dosing required only two visits, each with two injections at 30 minutes apart, followed by monthly maintenance injections. This schedule was accomplished with only one very mild systemic reaction. After 1 year of treatment, symptoms and medication use during the grass pollen season were reduced 34% and 40%, respectively, compared with placebo.

Recombinant technology allows production of the major allergens in large amounts. These may be similar in structure to the naturally occurring protein or there can be insertions, deletions, multiplication, or splitting of the molecules. In some cases, component peptides are derived, which may then be reformed into a mosaic protein²¹ or used in a cocktail for treatment.²² An example of a modified recombinant allergen is a folding variation of rBet v 1 that, after seven buildup and three maintenance doses, reduced symptoms in an environmental exposure chamber by nearly 80% compared with <20% for placebo.²³

Of all the recombinant products, the ones that have advanced the farthest in clinical development are the synthetic peptide immune-regulatory epitopes. These were initially developed as peptides derived from the major cat allergen Fel d 1.²⁴ The process of development included generating a number of Fel d 1–derived peptides, which represent T-lymphocyte epitopes. They were then selected for (1) showing binding affinity for 10 commonly expressed human leukocyte antigen-antigen D related molecules, (2) having the ability to induce proliferation and cytokine secretion from peripheral blood mononuclear cells of allergic donors, and (3) showing no reactivity with cat-specific immunoglobulin E.²⁴ These Fel d 1–derived peptides have been studied in an environmental chamber where those subjects who received four weekly intradermal injections of 6 nmol of peptides showed ~35% protection after 1 year and, without further treatment, 30% after 2 years compared with placebo.²⁵ A large phase III study has been completed, and results are anticipated in the spring of 2016. Similar peptide products derived from grass, ragweed, and house-dust mite allergens are in earlier phases of study.²⁶

Stimulation of Innate Immunity

DNA contains sequences termed CpG that, in mammals, are methylated, but, in bacteria and viruses, they are nonmethylated. As such, they react with Toll-like receptor 9 and release cytokines that direct the immune

response toward regulatory T cells (Treg) and T-helper (Th) cell type 1 orientation. There are two classes of CpG; the B type was investigated in the United States as immunostimulatory sequences bound to the major allergen of ragweed, Amb a 1. Initial studies at Johns Hopkins were impressive, with the clinical response persisting in the second year after a single course of six injections.²⁷ Subsequent large phase III studies did not produce favorable results, which resulted in discontinuation of the program. The A type CpG cannot be administered alone because it is rapidly degraded by enzymes that degrade deoxyribonucleic acids. This problem was overcome by coating the CpG with a protein coat derived from a bacteriophage.²⁸ Initial studies were favorable, even when it was administered without allergen in a series of seven injections to patients with allergic asthma in a placebo-controlled study.²⁸ However, a subsequent phase IIb study in patients with moderate-to-severe allergic asthma did not achieve statistically significant results, so development was discontinued by that manufacturer,²⁹ although some investigators remain optimistic about the product.³⁰

There is a line of products that are approved in some countries in Europe that combine several approaches for improving AIT. These products use an allergoid of the extract; adsorb it to tyrosine, which acts as to delay release from the site of injection; and also add monophosphoryl lipid A, a detoxified derivative of gram-negative bacterial cell walls. The preparation has been administered as four injections of increasing amounts at weekly intervals. A phase III study was conducted in the United States with the grass preparation in 1028 individuals.³¹ The overall benefit over placebo was only 13.4%, although *post hoc* analyses indicated that individuals with more severe symptoms or individuals exposed to higher levels of grass pollen fared better. The response to the ragweed pollen product was tested in an environmental exposure chamber.³² The ragweed allergoid adsorbed to tyrosine produced an improvement of only 6% compared with placebo, whereas the full product, with monophosphoryl lipid A, achieved a 20% improvement. Because the product has proven to be virtually free of adverse effects, some subsequent trials have added two additional injections, the first twice and the second four times the amount administered in the fourth injections. This has resulted in a considerably greater clinical response (Tim Higenbottam, M.D., DSc., Allergy Therapeutics, personal communication).

Suppression of the Th2 Response

Two approaches have been used to suppress the Th2 response in hopes that this will enhance the response to conventional SCIT. The first combined an anti-interleukin 4 monoclonal antibody with grass SCIT. Although there was improvement in both arms, there

was no additional improvement with the addition of the anti-interleukin 4.³³ The second approach, currently underway, is to add suppression of thymic stromal lymphopoietin with a monoclonal antibody to conventional SCIT with cat allergen extract (Immune Tolerance Network, ITN057AD, National Institutes of Health, Bethesda, MD). The results of this study are not expected until 2018.

Adjuvants

Substances that may enhance the development of Treg lymphocytes could enhance the response to conventional SCIT. A healthy microbiome in infants favors a normal balance between Th1 and Th2 lymphocytes.³⁴ It is possible that manipulation of the microbiome with probiotics in later life would have a similar effect. Studies are underway in this regard, but the results have not yet been published. Vitamin D has a number of immune-related actions, including induction of Tregs.³⁵ A study of the response to AIT in children was analyzed for the effect of vitamin D serum levels. Those children with normal levels had a significantly better response.³⁶ In a double-blind, placebo-controlled study in 50 children, the clinical response to SLIT grass tablets with or without 1000 units of vitamin D daily was compared.³⁷ After 5 months of treatment nasal and asthma symptoms during the grass pollen season were significantly lower in children who received the supplemental vitamin D compared with those who received the SLIT tablet alone.

CONCLUSION

The clinical efficacy of SCIT in treating allergic rhinitis, allergic asthma, and Hymenoptera venom sensitivity is well established. The appropriate doses of allergen extracts and the optimal duration of treatment have been determined in double-blind, placebo-controlled studies. Adequate courses of treatment led to modification of the underlying immune responses, which resulted in persisting improvement for a number of years after discontinuation. SLIT shares many of these features plus the advantages of far fewer systemic reactions and the convenience of home administration. However, with SLIT, there are the unresolved issues of effective dosing with liquid preparations and the efficacy of administering sublingually allergen extract mixes. Despite the attractive features of SCIT and SLIT, many appropriate patients decline treatment or, if they begin, stop long before they have completed a prescribed course of treatment.

A number of new approaches to AIT are being actively investigated, with the principal goal being to shorten the course in some cases down to just three to six administrations. Among the many approaches, those that are most promising are epicutaneous administration by patches; intralymphatic injections; recombinant modified allergens, including peptides derived

from T-lymphocyte epitopes, which simultaneously stimulate Th1 and/or Treg or suppress Th2 responses; or administration with adjuvants that stimulate Treg lymphocytes, such as probiotics or vitamin D.

REFERENCES

1. Sikora JM, and Tankersley MS. Perception and practice of sublingual immunotherapy among practicing allergists in the United States: A follow-up survey. *Ann Allergy Asthma Immunol* 110:194–197.e4, 2013.
2. Cox L, Nelson H, Lockett R, et al. Allergen immunotherapy: A practice parameter third update. *J Allergy Clin Immunol* 127(suppl. 1):S1–S55, 2011.
3. Nelson HS. Subcutaneous injection immunotherapy for optimal effectiveness. *Immunol Allergy Clin North Am*. 31:211–226, viii, 2011.
4. Pajno GB, Barberio G, De Luca F, et al. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy* 31:1392–1397, 2001.
5. Jacobsen L, Niggemann B, Dreborg S, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up of the PAT study. *Allergy* 62: 943–948, 2007.
6. Durham SR, Walker SM, Varga EM, et al. Long-term efficacy of grass-pollen immunotherapy. *N Engl J Med* 341:468–475, 1999.
7. Durham SR, and Penagos M. Sublingual or subcutaneous immunotherapy for allergic rhinitis? *J Allergy Clin Immunol* 137: 339–349.e10, 2016.
8. Marogna M, Spadolini I, Massolo A, et al. Long-lasting effects of sublingual immunotherapy according to its duration: A 15-year prospective study. *J Allergy Clin Immunol* 126:969–975, 2010.
9. Amar SM, Harbeck RJ, Sills M, et al. Response to sublingual immunotherapy with grass pollen extract: Monotherapy versus combination in a multi-allergen extract. *J Allergy Clin Immunol* 124:150–156.e1–5, 2009.
10. Kiel MA, Roder E, Gerth van Wijk R, et al. Real-life compliance and persistence among users of subcutaneous and sublingual allergen immunotherapy. *J Allergy Clin Immunol* 132:353–360.e2, 2013.
11. Nelson HS, Makatsori M, and Calderon MA. Subcutaneous immunotherapy and sublingual immunotherapy: Comparative efficacy, current and potential indications, and warnings—United States versus Europe. *Immunol Allergy Clin North Am* 36:13–24, 2016.
12. Senti G, and Kundig TM. Novel delivery routes for allergy immunotherapy. *Immunol Allergy Clin North Am* 36:25–37, 2016.
13. Senti G, von Moos S, Tay F, et al. Determinants of efficacy and safety in epicutaneous allergen immunotherapy: Summary of three clinical trials. *Allergy* 70:707–710, 2015.
14. Senti G, Prinz Vavricka BM, Erdmann I, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: A randomized controlled trial. *Proc Natl Acad Sci U S A* 105:17908–17912, 2008.
15. Hylander T, Larsson O, Petersson-Westin U, et al. Intralymphatic immunotherapy of pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled trial. *Respir Res* 17:10, 2016.
16. Patterson AM, Bonny AE, Shiels WE II, and Erwin EA. Three-injection intralymphatic immunotherapy in adolescents and young adults with grass pollen rhinoconjunctivitis. *Ann Allergy Asthma Immunol* 116:168–170, 2016.
17. Zaleska A, Eiwegger T, Soyer O, et al. Immune regulation by intralymphatic immunotherapy with modular allergen translocation Mat vaccine. *Allergy* 69:1162–1170, 2014.
18. Witten M, Malling HJ, Blom L, et al. Is intralymphatic immunotherapy ready for clinical use in patients with grass pollen allergy? *J Allergy Clin Immunol* 132:1248–1252.e5, 2013.
19. Henmar H, Lund G, Lund L, et al. Allergen, immunogenicity and dose-relationship of three intact allergen vaccines and four allergoid vaccines for subcutaneous grass pollen immunotherapy. *Clin Exp Immunol* 153:316–323, 2008.
20. Klimek L, Uhlig J, Mosges R, et al. A high polymerized grass pollen extract is efficacious and safe in a randomized double-blind, placebo-controlled study using a novel up-dosing cluster-protocol. *Allergy* 69:1629–1638, 2014.
21. Linhart B, Focke-Tejkl M, Weber M, et al. Molecular evolution of hypoallergenic hybrid protein for vaccination against grass pollen allergy. *J Immunol* 194:4008–4018, 2015.
22. Focke-Tejkl M, Weber M, Niespodziana K, et al. Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen allergy. *J Allergy Clin Immunol* 135:1207–1217.e1–11, 2015.
23. Meyer W, Narkus A, Salapatek AM, and Hafner D. Double-blind, placebo-controlled, dose-ranging study of new recombinant hypoallergenic Bet v 1 in an environmental exposure chamber. *Allergy* 68:724–731, 2013.
24. Worm M, Lee HH, Kleine-Tebbe J, et al. Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy. *J Allergy Clin Immunol* 127:89–97.e1–14, 2011.
25. Couroux P, Patel D, Armstrong K, et al. Fel d 1-derived synthetic peptide immune-regulatory epitopes show a long-term treatment effect in cat allergic subjects. *Clin Exp Allergy* 45:974–981, 2015.
26. Portfolio on Circassia. Available online at www.circassia.co.uk; accessed April 26, 2016.
27. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 355:1445–1455, 2006.
28. Beeh KM, Kannies F, Wagner F, et al. The novel TLR9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J Allergy Clin Immunol* 131:866–874, 2013.
29. Cytos Biotechnology LTD announces negative topline results of clinical trial with CYT003. New media release April 14, 2014. Available online at www.cytos.com; accessed April 26, 2016.
30. Kundig TM, Klimek L, Schendzielorz P, et al. Is the allergen really needed in allergy immunotherapy? *Curr Treat Options Allergy* 2:72–82, 2015.
31. DuBuske LM, Frew AJ, Horak F, et al. Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. *Allergy Asthma Proc* 32:239–247, 2011.
32. Patel P, Holdich T, Fischer von Weikersthal-Drachenberg KJ, and Humber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. *J Allergy Clin Immunol* 133:121–129.e1–2, 2014.
33. Chaker AM, Shamji MH, Dumitru FA, et al. Short-term subcutaneous grass pollen immunotherapy under the umbrella of anti-IL-r: A randomized controlled trial. *J Allergy Clin Immunol* 137:452–461, 2016.
34. Arrieta MC, Stiemsma LT, Amenyogbe N, et al. The intestinal microbiome in early life: Health and disease. *Front Immunol* 5:427, 2014.
35. Mirzakhani H, Al-Garawi A, Weiss ST, and Litonjua AA. Vitamin D and the development of allergic disease: How important is it? *Clin Exp Allergy* 45:114–125, 2015.
36. Majak P, Jerzynska J, Smejda K, et al. Correlation of vitamin D with Foxp3 induction and steroid-sparing effect of immunotherapy in asthmatic children. *Ann Allergy Asthma Immunol* 109:329–335, 2012.
37. Jerzynsak J, Stelmach W, Rychlik B, et al. The clinical effect of vitamin D supplementation combined with grass-specific sublingual immunotherapy in children with allergic rhinitis. *Allergy Asthma Proc* 37:105–114, 2016. □

Copyright of Allergy & Asthma Proceedings is the property of OceanSide Publications Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.