

actions to the KLH protein in our skin-immunized volunteers were highly antigen-specific in vivo T-cell–dependent responses to residual antigen that was left at the local site from the first injection, to which the host had a primary immune T-cell JMR. Then similar reactions were elicited to secondary skin-test exposures. Such reactions also include antigen-specific T-cell proliferation with distinct kinetics, reactions that now are ripe for more modern molecular analysis.

Recognition that responses to the mRNA Covid-19 vaccines resemble JMR and CBH reactions may lead to skin testing in patients and to other related studies to better understand SARS-CoV-2 infections. Perhaps so-called “long Covid” has a similar pathogenesis and could respond to treatments appropriate to JMR and CBH reactions. An example may be the improvement that was seen in patients with long Covid who were treated with combined antihistamines, since the source of histamine may be the basophils.⁵

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Disclosure forms provided by the author are available with the full text of this letter at NEJM.org.

1. Blumenthal KG, Freeman EE, Saff RR, et al. Delayed large local reactions to mRNA-1273 vaccine against SARS-CoV-2. *N Engl J Med* 2021;384:1273-7.
2. Askenase PW, Atwood JE. Basophils in tuberculin and “Jones-Mote” delayed reactions of humans. *J Clin Invest* 1976;58:1145-54.
3. Duckett Jones T, Mote JR. The phases of foreign protein sensitization in human beings. *N Engl J Med* 1934;210:120-3.
4. Richerson HB, Dvorak HF, Leskowitz S. Cutaneous basophil hypersensitivity. I. A new look at the Jones-Mote reaction, general characteristics. *J Exp Med* 1970;132:546-57.
5. Glynn P, Tahmasebi N, Gant V, Gupta R. Long COVID following mild SARS-CoV-2 infection: characteristic T cell alterations and response to antihistamines. *J Investig Med* 2021 October 05 (Epub ahead of print).

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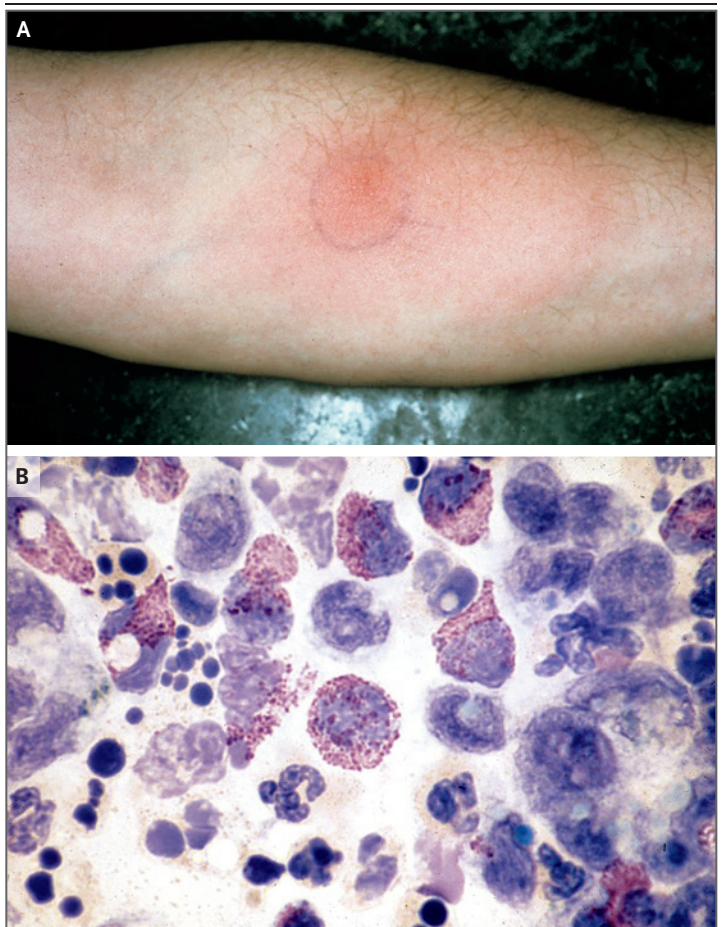


Figure 1. Cutaneous Basophil Hypersensitivity Response after Skin-Test Antigen Injection.

Shown are the results of a hypersensitivity reaction in a volunteer who received an intradermal skin-test injection of 20 μg of keyhole limpet hemocyanin (KLH) protein 1 week after priming with an intradermal immunization with 222 μg of KLH protein in a 1976 study.³ Panel A shows the delayed cutaneous erythematous and indurated JMR reaction on day 7 after the second test injection. Panel B shows the results of a Rebeck skin-window examination (in which the top layer of skin is scraped off to facilitate the identification of responding inflammatory cells) after 36 hours. In the numerous basophils that are shown, the cytoplasm is packed with metachromatic purple staining granules that obscure the nucleus. Polymorphonuclear neutrophils and mononuclear cells are also visible.

CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis

TO THE EDITOR: Maurer, in his editorial,¹ rightly acclaims the accomplishment of Gillmore et al. (August 5 issue),² who used a gene-editing technique that led to reduction of serum transthyretin (TTR) levels in six patients with hereditary

transthyretin amyloidosis (also called ATTR amyloidosis). However, although limiting or abolishing the production of this amyloidogenic precursor protein by hepatocytes could prevent further amyloid deposition, this achievement does not

address the critical issue of those patients whose organs have been compromised by extensive amyloid deposits. In ATTR and other systemic amyloidoses, only rarely, if ever, will the pathologic material resolve, given that it is composed of a self-protein and does not elicit an immune-mediated reaction that would lead to its removal. Knocking out TTR synthesis with the goal of “curing” hereditary or wild-type ATTR amyloidosis may be overly optimistic; curing the condition would also require a means to facilitate amyloidolysis. In this regard, passive immunotherapy with a fibril-specific monoclonal antibody³ can achieve this response through an Fc-mediated cellular reaction, as has been evidenced experimentally,⁴ and its therapeutic potential was shown in a phase 1a–b clinical trial.⁵

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Dr. Solomon reports being a consultant for, and having received stock options from, Caelum Biosciences. No other potential conflict of interest relevant to this letter was reported.

1. Maurer MS. Gene editing: a cure for transthyretin amyloidosis? *N Engl J Med* 2021;385:558-9.
2. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med* 2021; 385:493-502.
3. O’Nuallain B, Allen A, Kennel SJ, Weiss DT, Solomon A, Wall JS. Localization of a conformational epitope common to non-native and fibrillar immunoglobulin light chains. *Biochemistry* 2007;46:1240-7.
4. Hrnčić R, Wall J, Wolfenbarger DA, et al. Antibody-mediated resolution of light chain-associated amyloid deposits. *Am J Pathol* 2000;157:1239-46.
5. Edwards CV, Bhutani D, Mapara M, et al. One year follow up analysis of the phase 1a/b study of chimeric fibril-reactive monoclonal antibody 11-1F4 in patients with AL amyloidosis. *Amyloid* 2019;26:115-6.

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TO THE EDITOR: The study by Gillmore et al. proved the feasibility of the clinical application of therapy based on the clustered regularly interspaced short palindromic repeats and associated Cas9 endonuclease (CRISPR-Cas9) system for the treatment of patients with hereditary ATTR amyloidosis; however, a single guide RNA (sgRNA) targeting a specific mutation might be better than an sgRNA targeting both wild-type and mutant alleles, since it would preserve the normal function of TTR. Because the authors indicated that this approach can be adapted to treat other diseases with simple replacement of the sgRNA, we examined the gene-editing efficacy of muta-

tion-specific sgRNA design. Computational prediction algorithms¹ suggested that when an sgRNA targeting a specific mutation site is used for three missense mutations from this study, the gene-editing efficacy is similar to that achieved with the sgRNA targeting exon 2 of both wild-type and mutated TTR indiscriminately. In addition, the use of a mutation-specific sgRNA will make vitamin A supplementation unnecessary and will maintain normal thyroid function by preserving the normal activity of TTR.² We suggest that the major advantage of gene editing over previous RNA interference therapeutics³ can be strengthened if mutation-specific sgRNA rather than gene-based sgRNA is used.

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No potential conflict of interest relevant to this letter was reported.

1. Kim HK, Kim Y, Lee S, et al. SpCas9 activity prediction by DeepSpCas9, a deep learning-based model with high generalization performance. *Sci Adv* 2019;5(11):eaax9249.
2. Müller ML, Butler J, Heidecker B. Emerging therapies in transthyretin amyloidosis: a new wave of hope after years of stagnancy? *Eur J Heart Fail* 2020;22:39-53.
3. Adams D, Gonzalez-Duarte A, O’Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med* 2018;379:11-21.

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THE AUTHORS REPLY: Solomon questions whether ceasing production of the amyloid precursor is sufficient to reverse the course of systemic amyloidosis. Rim and colleagues speculate that a mutation-specific gene-editing approach could be developed and prove superior to the mutation-agnostic approach we described with NTLA-2001.

We agree that if an effective amyloidolytic agent were developed it might foster more rapid improvement than knockout of TTR alone, but we disagree with Solomon’s concern about the likelihood of reducing tissue amyloid burden after knockout. Serial scintigraphy¹ and cardiac magnetic resonance imaging² in all forms of amyloidosis amenable to treatment with protein-lowering agents have unequivocally shown that amyloid deposits are cleared in vivo. Reaching a new steady state in which the rate of natural amyloid clearance exceeds the rate of ongoing

amyloid formation requires a marked reduction in the concentration of the relevant circulating precursor protein.³ Since our gene-editing approach in ATTR amyloidosis led to serum TTR knockdown that exceeded 90% by day 28 in one of the first patients treated, it will be useful for future studies of NTLA-2001 to test the hypothesis that regression of ATTR amyloid can be achieved solely through a reduction in precursor protein.

Complete knockout of hepatic TTR is a more practical strategy and is likely to be more effective than a mutation-specific approach. Since 1990, liver transplantation has been used in the treatment of hereditary ATTR amyloidosis.⁴ With the organ transplant, circulating variant TTR is replaced with wild-type TTR. However, wild-type TTR continues to be deposited after transplantation in the hearts of patients with established variant ATTR cardiac amyloidosis, leading to poor outcomes.⁴ Furthermore, a mutation-specific strategy would require development of multiple independent drug candidates to address more than 100 different causative mutations for ATTR amyloidosis. Therefore, our approach provides a potential single solution for all hereditary forms of the disease and is likely to be more effective than the suggested mutation-specific strategy. Future gene-editing strategies in other diseases may benefit from a highly selective,

mutation-specific approach, although not in the case of ATTR amyloidosis, as Rim and colleagues suggest.

Finally, reductions in serum TTR caused by RNA-silencing therapies have not been shown to cause clinically significant thyroid or vitamin A deficiency. Patients treated with NTLA-2001 will undergo long-term monitoring for the detection of any clinically relevant effects.

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Since publication of their article, the authors report no further potential conflict of interest.

1. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with ¹²⁵I-labeled serum amyloid P component. *N Engl J Med* 1990;323:508-13.

2. Martinez-Naharro A, Abdel-Gadir A, Treibel TA, et al. CMR-verified regression of cardiac AL amyloid after chemotherapy. *JACC Cardiovasc Imaging* 2018;11:152-4.

3. Lachmann HJ, Goodman HJB, Gilbertson JA, et al. Natural history and outcome in systemic AA amyloidosis. *N Engl J Med* 2007;356:2361-71.

4. Stangou AJ, Hawkins PN, Heaton ND, et al. Progressive cardiac amyloidosis following liver transplantation for familial amyloid polyneuropathy: implications for amyloid fibrillogenesis. *Transplantation* 1998;66:229-33.

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Pegcetacoplan versus Eculizumab in PNH

TO THE EDITOR: Hillmen et al. (March 18 issue)¹ report that breakthrough hemolysis occurred in four patients (10%) receiving pegcetacoplan and in nine (23%) receiving eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria (PNH). They mention in the Discussion that breakthrough hemolysis was reported more often in patients who received eculizumab than in those who received pegcetacoplan and conclude that “a small subgroup of patients may require dose adjustments of pegcetacoplan after cessation of eculizumab; this warrants further study.” The primary problem is that this discussion unfolds without breakthrough hemolysis being defined anywhere in the main text.²

If we look at Table S4 in the Supplementary Appendix (available with the full text of their

article at NEJM.org), the severity of the hemolysis in patients in the pegcetacoplan group is totally different from that in the eculizumab group both in this trial and in the study conducted by Brodsky et al.² The hemolysis that was observed in the pegcetacoplan group may be similar to a major hemolytic attack as observed in untreated patients but is unlike the breakthrough hemolysis that is observed in patients receiving anticomplement drug treatment, such as eculizumab or ravulizumab. All four patients in the pegcetacoplan group whose data are in Table S4 meet the definition of breakthrough hemolysis (≥1 new or worsening symptom or sign of intravascular hemolysis, plus a lactate dehydrogenase [LDH] level of ≥2 times the upper limit of the normal range [ULN] after a re-