

## Clinical Communications

### High similarity between lentil and other lentil-like-proteins (dal) complicates recommendations on avoidance in lentil allergic patients

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#### Clinical Implications

- Current recommendations in lentil allergic patients suggest avoidance of all dal proteins. IgE binding patterns to different lentil-like legumes on the protein level do not provide sufficient information on cross-reactivity to guide clinical recommendations.

#### TO THE EDITOR:

Lentils together with chickpea are a common cause of IgE-mediated food-hypersensitivity reactions in children from the Mediterranean, Middle East, and some Asian countries.<sup>1</sup> In Spain, lentil is the fifth<sup>2</sup> and in Turkey the sixth<sup>3</sup> most common food allergen in the pediatric population. Because of changing preferences and alimentary trends, consumption of lentils and related legumes, often summarized under the Indian term of “dal,” has increased in the United States and Northern Europe. Allergy to lentils has been extensively studied, and allergenic peptides and epitopes are described.<sup>4,5</sup> In contrast to tree nut allergens, little is known about cross-reactivity among dal proteins to guide clinical management and recommendations for lentil allergic patients.

The predominant types of lentils consumed in Turkey are green and red lentils, and allergic reactions to either or both have been described.<sup>6</sup> As in other Mediterranean countries, lentils are a staple and weaning food in Spain with the predominant lentil consumed being the green lentil Armaña (Castellana) and the brown-red lentil Pardina. Lentil types consumed in the United States are more diverse due to a more heterogeneous population.

The present objective was to study the association between serum IgE binding patterns of lentil allergic patients on lentil-like (dal) proteins and clinical reaction histories to provide advice on selective avoidance.

Green lentil (*Lens culinaris* green), toor dal/pigeon pea (*Cajanus cajan*), mung dal/mung bean (*Vigna radiata*), urad dal/black gram (*Vigna mungo*), chana dal/chickpea (*Cicer arietinum*), mooth dal/moth bean (*Vigna aconitifolia*), and masoor dal/red lentil (*L. culinaris* red) extracts were prepared from cooked lentils as described elsewhere.<sup>7</sup> Sera from 3 lentil allergic children from Turkey, 3 lentil allergic children from Spain, and 3 lentil allergic children from the United States (all IgE > 0.35 kUA/L) and a negative control were used for immunoblotting (Table 1).

IgE reactivity to protein extracts was tested with individual sera from all subjects. Protein extracts (10 µg) were separated by SDS-PAGE (Nupage 4%-12% Zoom Gels; Invitrogen, ThermoFisher, Waltham, Mass) and transferred onto Immobilon-P membrane (Millipore, Billerica, Mass). The membranes were incubated with diluted sera (1:5 to 1:20 depending on

lentil-sIgE) for 1 hour at room temperature. Iodine-125-labeled goat antihuman IgE (DiaMed, Windham, Me) was used as the secondary antibody and incubated for 1 hour at room temperature. Membranes were exposed to Kodak Imaging Film (Carestream Health Inc., Rochester, NY) for 5-10 days.

To assess cross-reactivity banding and binding patterns, IgE levels and clinical reaction histories were compared among patients.

IgE binding patterns of lentil allergic patients' sera showed similarity among taxonomically and phenotypically similar dal proteins (mung dal and mooth dal; urad dal and toor dal; chana dal/chickpea).

IgE binding of lentil allergic patients was detected to all lentil-like proteins independent of specificity of sensitization. Binding regions coincided with defined locations of previously described allergens in *L. culinaris* (Len c 1, Len c 2, and Len c 3). IgE binding patterns to various lentil proteins did not allow for distinction between sensitization to various types of lentils. The group of Turkish patients presented with sensitization and described reactions to either green lentil (patient #2T) or red lentil (patient #3T) or both types of lentils (patient #1T). The reaction pattern is not reflected in IgE binding patterns on either red or green lentils. Detailed lentil subtype was not known for patients from Spain or the United States. The group of patients from Spain are sensitized to the types of lentil predominately consumed in Spain. Their IgE binding pattern did not differ significantly from binding patterns seen in the Turkish group. Similarly, the group of patients from the United States did not show distinguishable IgE binding patterns based on co-sensitization or clinical history (Figure 1). In our small sample, neither IgE levels nor co-sensitization to other legumes or pollens predicted the severity or type of reaction.

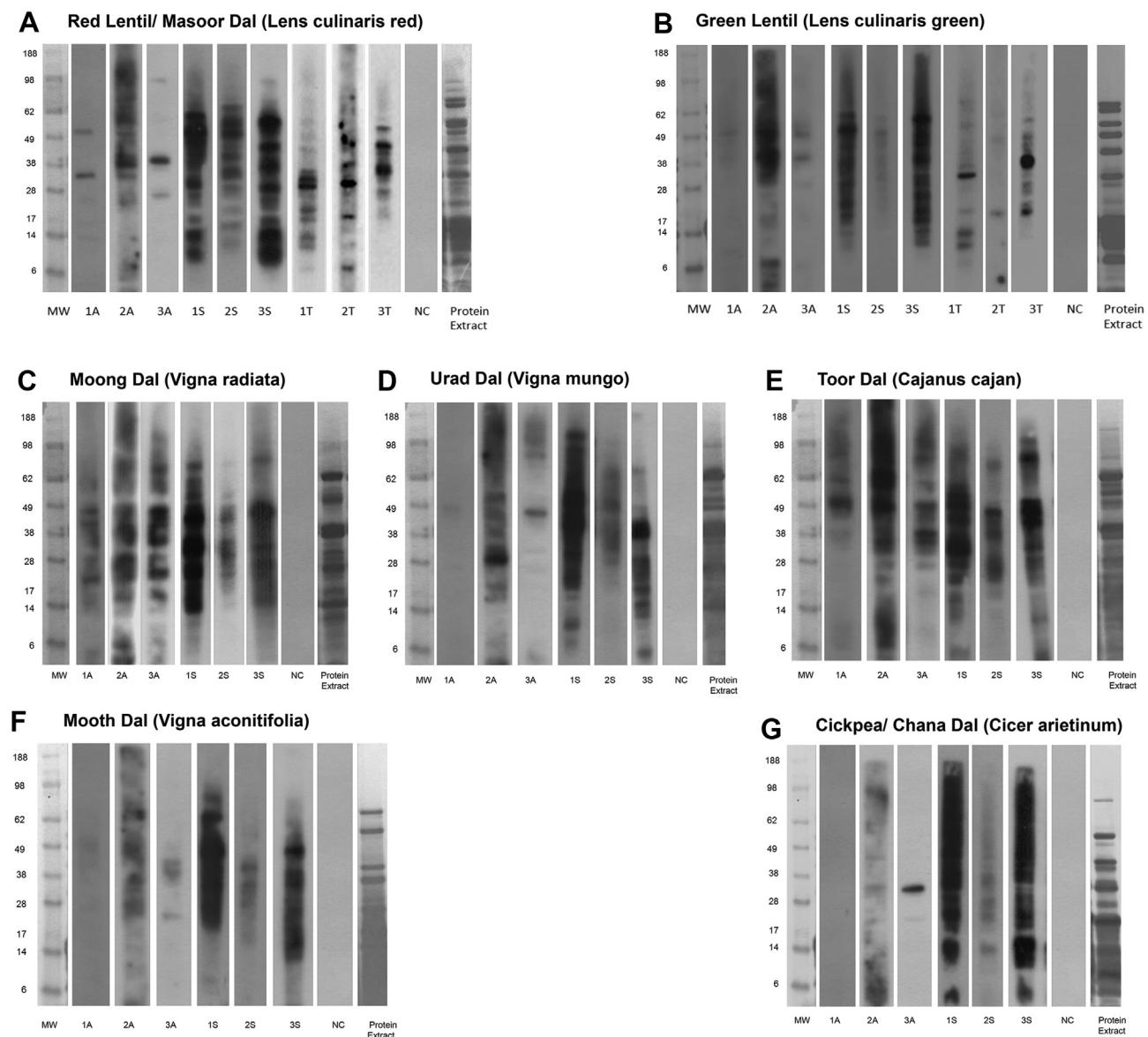
To assess the degree of relation between lentil (*L. culinaris*) and other legumes, the protein sequences were compared between lentil (Len c 1), chickpea provicilin and peanut (Ara h 1), all vicilin or provicilin proteins, and lentil (Len c 3) and peanut (Ara h 9), which both belong to the class of lipid transfer proteins. Among lentil-like (dal) proteins, the protein sequence has been described only for lentil (*L. culinaris*), with the definition of 3 major allergens (Len c 1, Len c 2, and Len c 3) and chickpea provicilin. IgE binding epitopes have been defined for Len c 1 previously. Sequence comparison between Len c 1 and chickpea provicilin revealed identity of 47.3% and consensus of 57.1% with more than 90% identity at the location of previously described Len c 1 IgE binding epitopes<sup>8</sup> (see Figure E1 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). Additionally, high degrees of identity were found comparing Len c 1 with other proteins from the vicilin family and Len c 3 with other lipid transfer proteins (see Table E1 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)).

Our results indicate a high degree of identity at the protein level between selected lentil-like (dal) proteins and other legumes. Despite or possibly because of the similarity noted at the protein level, no prediction about likelihood of tolerance versus reactivity could be made even in patients with symptoms due to distinct types of lentil. Protein sequences and IgE binding epitopes have not been described for all lentil-like (dal) proteins.<sup>9</sup>

**TABLE I.** Patient characteristics, lentil-specific IgE, and skin prick testing performed at the time of evaluation

| Patient ID (Country) | Age (y)/gender | Age of lentil allergy onset | Lentil-specific IgE | Skin prick test | Sensitization to lentil subtype | Symptoms with lentil exposure                | Pollen allergy | Other food allergies             |
|----------------------|----------------|-----------------------------|---------------------|-----------------|---------------------------------|----------------------------------------------|----------------|----------------------------------|
| 1T (Turkey)          | 4/F            | 1                           | 25.6                | Positive        | Red and green lentil            | Anaphylaxis                                  | Yes            | Chickpea                         |
| 2T (Turkey)          | 4/F            | 1                           | 4.37                | Positive        | Green lentil only               | Urticaria, then tolerated oral challenge     | No             | Chickpea                         |
| 3T (Turkey)          | 13.7/F         | 2.5                         | >100                | Positive        | Red lentil only                 | Anaphylaxis                                  | Yes            | Chickpea, pea, peanut, tree nuts |
| 1S (Spain)           | 5/F            | 2                           | >100                | Positive        | na                              | Anaphylaxis                                  | Yes            | Chickpea, pea, bean, soy         |
| 2S (Spain)           | 13/F           | 2                           | >100                | Positive        | na                              | Anaphylaxis                                  | Yes            | Chickpea, pea, bean              |
| 3S (Spain)           | 5/F            | 1                           | >100                | Positive        | na                              | Oral symptoms, urticaria                     | No             | Chickpea, pea, bean              |
| 1A (USA)             | 7/M            | 6.5                         | 6.87                | na              | na                              | Anaphylaxis                                  | Yes            | Peanut, chickpea                 |
| 2A (USA)             | 12/M           | 11                          | 41.5                | Positive        | na                              | Positive testing, tolerated oral challenge   | Yes            | Chickpea, soy                    |
| 3A (USA)             | 10/F           | na                          | 52.4                | Positive        | na                              | Oral symptoms, then tolerated oral challenge | Yes            | Peanut, chickpea, soy            |

na, not applicable/available.

**FIGURE 1.** Immunolabeling with polyclonal iodine-125-labeled antihuman IgE: labeling of all 9 patients (Turkish, Spanish, US) on red (A) and green (B) lentil extracts. (C-G), IgE binding patterns of Spanish and American patients on additional dal proteins.

Because specific IgE testing is limited to testing of green lentil (*L. culinaris* green) when using commercially available systems and prick-prick testing with individual legumes is not standardized, oral food challenge testing remains the only reliable method for predicting tolerance or reactivity to various lentils. Identification of the primary protein sequence and description of IgE binding epitopes together with competition and inhibition experiments will be necessary to guide clinical recommendations for allergic patients.

## CONCLUSIONS

Similar binding patterns among selected dal proteins point to high identity at the protein level that makes evaluation of IgE binding to discriminate clinical reactivity difficult. Clinical cross-reactivity is likely and has to be evaluated in more detail.

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|                                        |       |                                                                                                                                                                                                                              |
|----------------------------------------|-------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                        |       | 1                                                                                                                                                                                                                            |
| Len c 1.01 CAD87730                    | (1)   | -----SRS <sup>CO</sup> ENPFFIFKSN-----FQT <sup>Y</sup> YENE <sup>GH</sup> IRLLQR <sup>FD</sup> KR <sup>SK</sup> FEN <sup>Q</sup> NYRLLEYK <sup>SK</sup> P                                                                    |
| Chickpea provicilin_CAA36188           | (1)   | MIVRF <sup>SL</sup> PN <sup>EN</sup> DLKLTRSINRDGEILIP <sup>IF</sup> II <sup>IS</sup> VS <sup>IS</sup> NGASRE <sup>FD</sup> GI <sup>SK</sup> VE <sup>FL</sup> SLGFNTV <sup>SI</sup> A                                        |
| Consensus                              | (1)   | S DNEN K I N FD S I L S                                                                                                                                                                                                      |
|                                        |       | 71                                                                                                                                                                                                                           |
| Len c 1.01 CAD87730                    | (56)  | HT <sup>IF</sup> IPQF <sup>TD</sup> ADF <sup>LV</sup> LSGKA <sup>LT</sup> V <sup>NS</sup> NDNRNSFN <sup>ER</sup> GDTIK <sup>PA</sup> GT <sup>AY</sup> LANRDDNED <sup>RV</sup> LD---L                                         |
| Chickpea provicilin_CAA36188           | (71)  | LH <sup>IG</sup> QDC <sup>SR</sup> HHCH <sup>VE</sup> ERGCEV <sup>SY</sup> FL <sup>OT</sup> VVLEVLK <sup>LR</sup> TFVEPL <sup>EK</sup> TN <sup>TV</sup> FVLEKSLKV <sup>RL</sup> K <sup>K</sup> RI <sup>LI</sup>              |
| Consensus                              | (71)  | I L S IL I LNS L R L I LRL D L                                                                                                                                                                                               |
|                                        |       | 141                                                                                                                                                                                                                          |
| Len c 1.01 CAD87730                    | (123) | ATP <sup>NR</sup> PQQLQSFL <sup>LS</sup> GT <sup>QN</sup> QPSF <sup>LS</sup> FSKNILEA <sup>NT</sup> Y <sup>IE</sup> IE <sup>IV</sup> LVLEE <sup>QEQ</sup> K <sup>SQ</sup> HR <sup>SL</sup> RD <sup>K</sup> ROI <sup>EL</sup> |
| Chickpea provicilin_CAA36188           | (141) | LIG <sup>SH</sup> KLRPRKQF <sup>LS</sup> TKSGNRA <sup>II</sup> ILMIEFLL <sup>ER</sup> II <sup>DE</sup> IE <sup>IV</sup> LVLEE <sup>QEQ</sup> K <sup>PK</sup> QR <sup>RG</sup> HP <sup>KD</sup> QCS <sup>QS</sup>             |
| Consensus                              | (141) | I I LS T L A AF D DEIEKVLLEE <sup>QEQ</sup> K RR KDK Q S                                                                                                                                                                     |
|                                        |       | 211                                                                                                                                                                                                                          |
| Len c 1.01 CAD87730                    | (193) | EE---DVIVK <sup>VS</sup> REQIEELSKNAKSSSKSVSSESEPFNLSRNPIYSNK <sup>EG</sup> FFEITPEKNPOLQDLD                                                                                                                                 |
| Chickpea provicilin precursor_CAA36188 | (210) | SQQEA <sup>DV</sup> IVK <sup>IS</sup> REQIEELSKNAKSSSKSVSSESEPFNLSRNPIYSNK <sup>YCN</sup> FFEITPEKNPOLQDLD                                                                                                                   |
| Consensus                              | (211) | N DVIVKISREQIEELSKNAKSSSKSVSSESEPFNLSRNPIYSNKFG FFEITPEKNPOLQDLD                                                                                                                                                             |
|                                        |       | 281                                                                                                                                                                                                                          |
| Len c 1.01 CAD87730                    | (259) | IF <sup>NS</sup> VEI <sup>EG</sup> SLLLP <sup>NS</sup> RAI <sup>VI</sup> TVNEGKG <sup>EL</sup> VG <sup>Q</sup> RNEN <sup>Q</sup> QEQ <sup>EE</sup> EN <sup>EE</sup> EGQ <sup>EE</sup> ETTK <sup>QV</sup> QR <sup>IR</sup>    |
| Chickpea provicilin precursor_CAA36188 | (280) | IS <sup>NS</sup> VEI <sup>EG</sup> SLLLP <sup>NS</sup> RAI <sup>VI</sup> TVNEGKG <sup>EL</sup> VG <sup>L</sup> RNENE <sup>D</sup> EN <sup>KE</sup> DEE <sup>EE</sup> ---EED <sup>RK</sup> V <sup>QV</sup> QR <sup>EQ</sup>   |
| Consensus                              | (281) | I LNSVEI EGSLLLP FNSRA VIL VNEGKG ELVG RNEN QENK E DEE EED QVQRF                                                                                                                                                             |
|                                        |       | 329                                                                                                                                                                                                                          |
| Len c 1.01 CAD87730                    | (329) | ARLS <sup>GD</sup> VLVIPAGHPVAINASSDLNLIGFGINAKNNQRNFLAGEDNVISQIQRPVKELAFPGSSREVD                                                                                                                                            |
| Chickpea provicilin precursor_CAA36188 | (347) | ARLS <sup>GD</sup> VLVIPATHF <sup>SI</sup> INASSDLFLIGFGINAKNNQRNFLAGEDNVISQIQRPVKELAFPGSSREVD                                                                                                                               |
| Consensus                              | (351) | ARLS GDVLVIPA HP AINASSDL LIGFGINA NNQRNFLAGEDNVISQIQRPVKELAFPGSA EVD                                                                                                                                                        |
|                                        |       | 399                                                                                                                                                                                                                          |
| Len c 1.01 CAD87730                    | (399) | RLL <sup>TN</sup> Q <sup>Q</sup> SHFANAQPL <sup>QI</sup> IE-----                                                                                                                                                             |
| Chickpea provicilin precursor_CAA36188 | (417) | RLL <sup>KN</sup> Q <sup>Q</sup> SHFANAQPL <sup>QK</sup> IEESQKIRIPLSSILGGF                                                                                                                                                  |
| Consensus                              | (421) | RLL NQ <sup>Q</sup> SHFANAQPL Q D                                                                                                                                                                                            |

■ Epitope 1 (EEGQEEETTKQVQRYRARLSPGDVLVIPAGHPVAIN)

■ Epitope 2 (PAGHPVAINASSDLNLIGFGINAKNNQ)

■ Epitope 3 (LIGFGINAKNNQRNFLAGEDNVIS)

■ Epitope 4 (EEDNVISQIQRPVKELAFPGSSREVD)

**FIGURE E1.** Sequence comparison of lentil (Len c 1) and chickpea provicilin. The IgE binding epitopes defined on Len c 1 are partially overlapping. They are located in regions that show high identity to chickpea provicilin.

**TABLE E1.** Comparison of the protein sequence of lentil vicilin with other vicilins described in tree nuts or legumes and lentil (lipid transfer protein, LTP) with peanut or tree nut LTP showing high degree of identity and consensus

| % Identity<br>% Consensus   | Chickpea<br>provicilin | Pis s 1<br>(pea vicilin) | Pis s 2 (pea<br>convicilin) | Ara h1 (peanut<br>vicilin) | Cor a 11<br>(hazelnut<br>vicilin) | Ara h 9<br>(peanut LTP) | Cor a 8<br>(hazelnut LTP) | Jug r 3<br>(walnut LTP) |
|-----------------------------|------------------------|--------------------------|-----------------------------|----------------------------|-----------------------------------|-------------------------|---------------------------|-------------------------|
| Len c 1<br>(lentil vicilin) | 47.4<br>90             | 90<br>93.1               | 45<br>50.7                  | 33.4<br>42.4               | 31.3<br>41.6                      |                         |                           |                         |
| Len c 3<br>(lentil LTP)     |                        |                          |                             |                            |                                   | 52.5<br>61.9            | 49.2<br>64.4              | 52.5<br>65              |