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**Table S1.** Demographics profile and mean serum IgE levels of the AR patients.

|  |  |
| --- | --- |
| Variables | Frequency, n (%) |
| Gender  Male  Female | 25 (43.86%)  32 (56.14%) |
| Age (years)  Mean | 24.58 ± 12.19\* |
| Serum IgE (kU/L)  *D. pteronyssinus*  D. farinae  B. tropicalis  Total IgE (kU/L) | 29.30 ± 34.81\*  28.73 ± 33.90\*  19.97 ± 32.39\*  599.30\* |

*Note*. Data was expressed as mean ± standard deviation (S.D.). \*Average of 78 Malay AR patients.

**Table S2.** List of predicted CD4+ T cell epitopes on Der p 1 allergen.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Peptide sequences** | **Position** | **No. of binding HLA allele(s)** | **No of binding HLA serotype(s)** | **Cytokine-inducing capability** | | | **Status of allergenicity** |
| **IL-4** | **IL-10** | **IFN-γ** |
| T1 | MKIVLAIASLLALSA | 1 – 15 | 6 | 4 | - | - | ✓ | Non-allergenic |
| T2 | AIASLLALSAVYARP | 6 – 20 | 10 | 5 | - | ✓ | ✓ | Non-allergenic |
| T3 | SLLALSAVYARPSSI | 9 – 23 | 1 | 1 | - | ✓ | ✓ | Non-allergenic |
| T4 | KKAFNKSYATFEDEE | 30 – 44 | 1 | 1 | ✓ | - | - | Non-allergenic |
| T5 | NKSYATFEDEEAARK | 34 – 48 | 7 | 3 | ✓ | - | - | Allergenic |
| T6 | RKNFLESVKYVQSNG | 47 – 61 | 7 | 2 | ✓ | ✓ | ✓ | Non-allergenic |
| T7 | FLESVKYVQSNGGAI | 50 – 64 | 9 | 2 | ✓ | ✓ | ✓ | Non-allergenic |
| T8 | SVKYVQSNGGAINHL | 53 – 67 | 5 | 3 | ✓ | - | - | Non-allergenic |
| T9 | DLSLDEFKNRFLMSA | 69 – 83 | 8 | 1 | ✓ | - | - | Allergenic |
| T10 | FKNRFLMSAEAFEHL | 75 – 89 | 2 | 2 | ✓ | ✓ | - | Allergenic |
| T11 | AEAFEHLKTQFDLNA | 83 – 97 | 8 | 4 | ✓ | - | - | Allergenic |
| T12 | FEHLKTQFDLNAETN | 86 – 100 | 2 | 1 | ✓ | - | - | Allergenic |
| T13 | NAPAEIDLRQMRTVT | 107 – 121 | 4 | 2 | ✓ | - | - | Non-allergenic |
| T14 | IDLRQMRTVTPIRMQ | 112 – 126 | 4 | 2 | ✓ | ✓ | ✓ | Allergenic |
| T15 | LRQMRTVTPIRMQGG | 114 – 128 | 2 | 2 | ✓ | ✓ | ✓ | Allergenic |
| T16 | IRMQGGCGSCWAFSG | 123 – 137 | 1 | 1 | ✓ | - | - | Non-allergenic |
| T17 | WAFSGVAATESAYLA | 133 – 147 | 3 | 3 | - | - | ✓ | Allergenic |
| T18 | FSGVAATESAYLAYR | 135 – 149 | 5 | 2 | - | - | ✓ | Non-allergenic |
| T19 | ESAYLAYRNQSLDLA | 142 – 156 | 13 | 3 | - | - | ✓ | Non-allergenic |
| T20 | PRGIEYIQHNGVVQE | 175 – 189 | 9 | 3 | ✓ | - | - | Allergenic |
| T21 | ESYYRYVAREQSCRR | 189 – 203 | 14 | 5 | ✓ | ✓ | - | Allergenic |
| T22 | ISNYCQIYPPNVNKI | 211 – 225 | 1 | 1 | ✓ | ✓ | ✓ | Non-allergenic |
| T23 | NKIREALAQTHSAIA | 223 – 237 | 1 | 1 | ✓ | ✓ | - | Allergenic |
| T24 | ALAQTHSAIAVIIGI | 228 – 242 | 3 | 1 | - | ✓ | ✓ | Non-allergenic |
| T25 | HSAIAVIIGIKDLDA | 233 – 247 | 2 | 2 | - | - | - | Non-allergenic |
| T26 | IAVIIGIKDLDAFRH | 236 – 250 | 8 | 5 | - | - | - | Allergenic |
| T27 | DAFRHYDGRTIIQRD | 246 – 260 | 7 | 2 | ✓ | ✓ | - | Allergenic |
| T28 | QPNYHAVNIVGYSNA | 264 – 278 | 5 | 2 | ✓ | ✓ | ✓ | Allergenic |
| T29 | AVNIVGYSNAQGVDY | 269 – 283 | 14 | 3 | ✓ | ✓ | ✓ | Allergenic |
| T30 | AQGVDYWIVRNSWDT | 278 – 292 | 3 | 2 | ✓ | - | - | Non-allergenic |
| T31 | GYGYFAANIDLMMIE | 298 – 312 | 10 | 8 | ✓ | - | ✓ | Allergenic |
| T32 | NIDLMMIEEYPYVVI | 305 – 319 | 2 | 1 | ✓ | - | - | Allergenic |

*Note*. ( - ) indicates NON-INDUCER whereas ( ✓ ) indicates POSITIVE INDUCER.

**Table S3.** List of predicted linear B cell epitopes on Der p 1 allergen.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Peptide sequences** | **Position** | **Epitope prediction (IgPred)** | | | **Epitope mapping (IgPred)** | | **Elicited antibodies and their corresponding immunogenic potencies (Bcipep)** | | | | **Status of allergenicity** |
| **IgG** | **IgE** | **IgA** | **IgG** | **IgE** | **IgG** | **Potency** | **IgE** | **Potency** |
| B1 | KSYATFEDEEAARKNF | 35 – 50 | ✓ | - | ✓ | \* | \* | \* | \* | \* | \* | Allergenic |
| B2 | NAETNACSINGNAPA | 96 – 110 | - | ✓ | - | ✓ | - | \* | \* | ✓ | Immunodominant | Allergenic |
| B3 | QMRTVTPIRMQGGCGS | 116 – 131 | - | ✓ | ✓ | \* | \* | \* | \* | \* | \* | Allergenic |
| B4 | TPIRMQGGCGSCWAF | 121 – 135 | - | - | - | \* | \* | \* | \* | \* | \* | Allergenic |
| B5 | IEYIQHNGVVQESYY | 178 – 192 | - | ✓ | ✓ | ✓ | ✓ | \* | \* | ✓ | Immunogenic | Allergenic |
| B6 | GVVQESYYRYVAREQS | 185 – 200 | - | ✓ | - | \* | \* | \* | \* | ✓ | Immunogenic | Allergenic |
| B7 | YVAREQSCRRPNAQR | 194 – 208 | - | ✓ | - | - | ✓ | \* | \* | \* | \* | Allergenic |
| B8 | EQSCRRPNAQRFGISN | 198 – 213 | - | ✓ | - | - | ✓ | \* | \* | \* | \* | Allergenic |
| B9 | FGISNYCQIYPPNVNK | 209 – 224 | - | ✓ | ✓ | - | ✓ | \* | \* | ✓ | Immunogenic | Allergenic |
| B10 | YCQIYPPNVNKIREA | 214 – 228 | - | ✓ | ✓ | - | ✓ | \* | \* | ✓ | Immunogenic | Allergenic |
| B11 | HYDGRTIIQRDNGYQP | 250 – 265 | ✓ | ✓ | ✓ | \* | \* | \* | \* | \* | \* | Allergenic |
| B12 | VNIVGYSNAQGVDYW | 270 – 284 | ✓ | ✓ | - | ✓ | - | ✓ | Immunodominant | ✓ | Immunogenic | Allergenic |
| B13 | AQGVDYWIVRNSWDTN | 278 – 293 | ✓ | ✓ | - | ✓ | ✓ | ✓ | Immunodominant | ✓ | Immunogenic | Non-allergenic |

*Note*. ( - ) indicates NON-INDUCER, ( ✓ ) indicates POSITIVE INDUCER and ( \* ) indicates DATA NOT AVAILABLE.

**Table S4.** Sequence similarities between the predicted T and B cell epitopes and their immune properties.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **T cell epitopes** | **B cell epitopes** | **Percentage similarity** | **Corresponding HLA serotype(s)** | **Predicted antibodies** | **Immunogenic potencies (Bcipep)** |
| N**KSYATFEDEEAARK** | **KSYATFEDEEAARK**NF | 82.35% | DR10  DR15  DR16 | IgG and IgA | Unknown |
| LR**QMRTVTPIRMQGG** | **QMRTVTPIRMQGG**CGS | 72.22% | DR1  DR9 | IgE and IgA | Unknown |
| **IRMQGGCGSCWAF**SG | TP**IRMQGGCGSCWAF** | 76.47% | DR1 | None | Unknown |
| PRG**IEYIQHNGVVQE** | **IEYIQHNGVVQE**SYY | 66.67% | DR11  DR13  DR15 | IgE and IgA | Immunogenic IgE inducer |
| **ESYYRYVAREQS**CRR | GVVQ**ESYYRYVAREQS** | 63.16% | DR8  DR11  DR13  DR14  DR16 | IgE only | Immunogenic IgE inducer |
| **ISNYCQIYPPNVNK**I | FG**ISNYCQIYPPNVNK** | 82.35% | DR1 | IgE and IgA | Immunogenic IgE inducer |
| ISN**YCQIYPPNVNKI** | **YCQIYPPNVNKI**REA | 66.67% |
| A**VNIVGYSNAQGVDY** | **VNIVGYSNAQGVDY**W | 87.50% | DR4  DR7  DR15 | IgG and IgE | Immunodominant IgG inducer and immunogenic IgE inducer |
| **AQGVDYWIVRNSWDT** | **AQGVDYWIVRNSWDT**N | 93.75% | DR4 DR13 |

*Note*. Overlapping sequences between T and B cell epitopes highlighted in bold. Only moderate to high similarity matches were shown.

**Table S5.** Correlation analysis between HDM-specific IgE levels and HLA-DR groups.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **HLA-DR serotypes** | **Independent samples t-test** | | |  | **Multiple linear regression** | |
| **Mean patient serum levels (log)** | | ***p*-value** |  | **Standardized beta coefficients (β)** | ***p*-value** |
|  | **HLA (+)** | **HLA (-)** |  |  |  |  |
| **DR1** |  |  |  |  |  |  |
| Total IgE | 5.56 | 5.07 | NS |  | -0.880 | NS |
| *Derp* sIgE | 0.85 | 1.09 | NS |  | -0.240 | NS |
| *Derf* sIgE | 0.25 | 0.99 | NS |  | -0.256 | NS |
| *Blot* sIgE | 1.27 | 0.56 | NS |  | -0.790 | NS |
| **DR4** |  |  |  |  |  |  |
| Total IgE | 4.52 | 5.14 | NS |  | -0.371 | NS |
| *Derp* sIgE | -0.01 | 1.15 | NS |  | -0.193 | NS |
| *Derf* sIgE | 0.32 | 1.01 | NS |  | -0.144 | NS |
| *Blot* sIgE | 0.92 | 0.56 | NS |  | 0.015 | NS |
| **DR7** |  |  |  |  |  |  |
| Total IgE | 3.18 | 5.25 | NS |  | -0.627 | NS |
| *Derp* sIgE | -3.36 | 1.34 | 0.009 |  | -0.464 | 0.026 |
| *Derf* sIgE | -3.71 | 1.24 | 0.008 |  | -0.444 | 0.040 |
| *Blot* sIgE | -4.26 | 0.86 | 0.004 |  | -0.503 | 0.022 |
| **DR8** |  |  |  |  |  |  |
| Total IgE | 3.56 | 5.15 | NS |  | -0.225 | NS |
| *Derp* sIgE | 0.76 | 1.10 | NS |  | -0.073 | NS |
| *Derf* sIgE | 0.87 | 0.98 | NS |  | -0.760 | NS |
| *Blot* sIgE | 1.32 | 0.54 | NS |  | 0.023 | NS |
| **DR10** |  |  |  |  |  |  |
| Total IgE | 5.09 | 5.82 | NS |  | -0.199 | NS |
| *Derp* sIgE | -0.25 | 1.12 | NS |  | -0.085 | NS |
| *Derf* sIgE | -0.48 | 1.01 | NS |  | -0.093 | NS |
| *Blot* sIgE | -0.43 | 0.61 | NS |  | -0.041 | NS |
| **DR11** |  |  |  |  |  |  |
| Total IgE | 5.52 | 4.93 | NS |  | 0.195 | NS |
| *Derp* sIgE | 0.27 | 1.31 | NS |  | -0.143 | NS |
| *Derf* sIgE | 0.84 | 1.22 | NS |  | -0.153 | NS |
| *Blot* sIgE | 0.22 | 0.68 | NS |  | -0.774 | NS |
| **DR12** |  |  |  |  |  |  |
| Total IgE | 5.20 | 5.06 | NS |  | 0.183 | NS |
| *Derp* sIgE | 0.55 | 1.23 | NS |  | -0.228 | NS |
| *Derf* sIgE | 0.64 | 1.06 | NS |  | -0.166 | NS |
| *Blot* sIgE | 0.72 | 0.54 | NS |  | -0.920 | NS |
| **DR13** |  |  |  |  |  |  |
| Total IgE | 4.01 | 5.35 | NS |  | -0.414 | NS |
| *Derp* sIgE | 0.74 | 1.20 | NS |  | -0.147 | NS |
| *Derf* sIgE | 0.63 | 1.08 | NS |  | -0.121 | NS |
| *Blot* sIgE | -0.36 | 0.88 | NS |  | -0.299 | NS |
| **DR14** |  |  |  |  |  |  |
| Total IgE | 5.50 | 5.02 | NS |  | -0.265 | NS |
| *Derp* sIgE | 2.31 | 0.89 | NS |  | 0.400 | NS |
| *Derf* sIgE | 2.09 | 0.80 | NS |  | 0.340 | NS |
| *Blot* sIgE | 1.84 | 0.38 | NS |  | 0.102 | NS |
| **DR15** |  |  |  |  |  |  |
| Total IgE | 0.54 | 4.93 | NS |  | 0.057 | NS |
| *Derp* sIgE | 1.91 | 0.52 | NS |  | 0.112 | NS |
| *Derf* sIgE | 1.65 | 0.51 | NS |  | 0.129 | NS |
| *Blot* sIgE | 0.59 | 0.57 | NS |  | -0.221 | NS |
| **DR16** |  |  |  |  |  |  |
| Total IgE | 1.02 | 1.47 | NS |  | -0.160 | NS |
| *Derp* sIgE | 1.14 | 2.50 | NS |  | -0.294 | NS |
| *Derf* sIgE | 1.38 | 2.59 | NS |  | -0.319 | NS |
| *Blot* sIgE | 1.53 | 2.53 | NS |  | -0.374 | NS |
| **DR17** |  |  |  |  |  |  |
| Total IgE | 0.72 | 1.55 | NS |  | -0.112 | NS |
| *Derp* sIgE | 1.68 | 2.59 | NS |  | 0.145 | NS |
| *Derf* sIgE | 1.73 | 2.68 | NS |  | 0.145 | NS |
| *Blot* sIgE | 1.46 | 2.61 | NS |  | 0.165 | NS |
| **DR18** |  |  |  |  |  |  |
| Total IgE | 3.73 | 5.27 | NS |  | 0.064 | NS |
| *Derp* sIgE | -1.29 | 1.13 | NS |  | -0.171 | NS |
| *Derf* sIgE | -1.41 | 1.26 | NS |  | -0.159 | NS |
| *Blot* sIgE | -0.98 | 0.77 | NS |  | 0.035 | NS |

*Note*. ( NS ) indicates NOT SIGNIFICANT.

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**Figure S1.** Type of cytokines induced by the predicted T cell epitopes of Der p 1 (n = 32).

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**Figure S2.** Allergenicity status of the predicted T cell epitopes of Der p 1 (n = 32).

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**Figure S3.** Type of antibodies induced by the predicted B cell epitopes of Der p 1 (n = 13).

**Methodology**

Prediction of T and B cell epitopes of Der p 1

The full amino acid sequence of Der p 1 allergen was uploaded into three different HLA-peptide prediction software: TepiTool, NetMHCIIpan 4.0 and PREDIVAC for recognition of T cell epitopes. A panel of *HLA-DRB1* alleles, identified to at least 4-digit resolution, found in the Malay population was selected for this analysis.1 The cutoff point used for TepiTool prediction was 5.00 whereas for NetMHCIIpan 4.0, a value of 2.00 was set. For PREDIVAC, a score of more than 80.00% was used for peptide selection. The generated lists of peptides from the three web programs were compared against one another for sequence similarities. Sequences that matched in at least 2 out of the 3 web programs were taken as potential CD4+ T cell epitopes in this study. Similarly, the webtools ABCPred, BCPREDS and BepiPred 2.0 were used for identification of linear B cell epitopes. For ABCPred, a score of 0.80 was taken as the minimum point value for peptide selection whereas a threshold of 0.550 was used for BepiPred 2.0. A 2/3 software consensus approach was taken to establish the final list of B cell peptides. The Needleman-Wunsch global alignment tool from NCBI BLAST was used for identification of percentage of sequence similarities between T and B cell peptides.

**Reference**

1. Tan LK, Farid BM, Salsabil S, et al. HLA-A, -B, -C, -DRB1 and -DQB1 alleles and haplotypes in 951 Southeast Asia Malays from Peninsular Malaysia. *Hum Immunol*. 2016;77(10):818-819. doi:10.1016/j.humimm.2016.06.022