Results
CLINICAL, PARASITOLOGICAL AND IMMUNOLOGICAL
STATUS OF THE STUDY POPULATION:

The endemic normal individuals and the asymptomatic microfilaria carriers were selected from few villages from Khurda district of Orissa state, India, which falls within 20° latitude and 86° longitude and is highly endemic for bancroftian filariasis. After surveying the population for a history of filariasis, they were given a physical examination by a clinician well versed with the signs and symptoms of lymphatic filariasis. Flowchart-1 shows the screening procedure followed for selecting the study population. Individuals showing chronic or acute filarial pathology were categorized as symptomatic individuals. Out of the total 458 individuals surveyed 87 individuals had either chronic or acute symptoms of lymphatic filariasis. We did not come across any TPE patients in our survey. All the asymptomatic individuals were then screened for the presence of parasite in the blood, by taking approximately 20μl of finger prick blood, collected between 22:00hrs and 02:00hrs., and a thick smear was made. The smears were stained and examined microscopically for the presence of microfilaria. Out of the total 371 individuals screened for microfilaria 53 were found positive. Out of the remaining 318 amicrofilaremic, 81 individuals and 17 microfilaremic individuals, who were willing to participate in the study were bled for 2.0ml of venous blood, after getting an informed consent from them. All these samples were tested for Circulatory Filarial Adult (CFA) antigen by Og4C3 ELISA and 100 units of antigen/ml of sera was taken as the cut-off value as recommended by the manufacturer. All the microfilaremic and 7 of
### Results

**Total No. of individuals screened from Khurda, Orissa**

<table>
<thead>
<tr>
<th>No. of individuals screened from Khurda, Orissa</th>
<th>Age: 14-58yrs.</th>
<th>M:265, F:193</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No Symptoms of LF</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td>371 (age:14-53yrs.)</td>
<td>(M:217, F:154)</td>
</tr>
<tr>
<td><strong>Symptoms of LF</strong></td>
<td>87 (age:23-58yrs.)</td>
<td>(M:48, F:39)</td>
</tr>
<tr>
<td><strong>Chronic</strong></td>
<td>56 (age:29-58yrs.)</td>
<td>(M:31, F:25)</td>
</tr>
<tr>
<td><strong>Acute</strong></td>
<td>31 (age:23-47yrs.)</td>
<td>(M:17, F:14)</td>
</tr>
</tbody>
</table>

**Microfilaremic**

- (M:46, F:7)
- 53 (age:14-51yrs.)

**Amicrofilaremic**

- (M:171, F:147)
- 318 (age:14-53yrs)

**Willing to participate in the study**<sup>**</sup>

- 16 (age:16-48yrs.)
  - (M:9, F:7)
- 81 (age:18-53yrs.)
  - (M:52, F:29)

**Og4C3 ELISA**

- **All Positive**
  - 74 (age:18-53yrs.)
    - (M:45, F:29)
  - All High ASM

- **Negative**
  - 7 (age:26-43yrs.)
    - (M:7, F:0)
  - All Low

**IgG4 titre to BmA**

**Longitudinal Follow up**

**Putative EN**

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**Flowchart - 1:** Screening of population living in the study area for identification of EN & ASM individuals

* Lymphatic Filariasis

** **
the microfilaremic individuals were found to be positive for CFA. The parasite specific IgG4 titre was determined in the sera of all the microfilaremic individuals and the CFA negative microfilaremic individuals by ELISA against *B. malayi* adult soluble antigen (BmA). All the 74 microfilaremic individuals had low parasite specific IgG4 titre, while all the microfilaremic individuals had high parasite specific IgG4 titres against BmA.

The 16 asymptomatic microfilaremic (ASM) individuals were again given a clinical examination by a clinician for the presence of other diseases besides lymphatic filariasis. 5 ASM individuals were found to be suffering from other diseases like anemia, kidney problems, tuberculosis and chronic asthma, and hence had to be dropped out of the study and the remaining 11 ASM individuals were included in the study. The epidemiological characteristic of the study population is summarized in Table-2. All the 74 asymptomatic microfilaremic individuals were followed up longitudinally at a regular interval of 6 months for 3 years. During each follow up these individuals were tested for all the above mentioned criteria and at the end of 3 years those individuals who continued to fulfill all the selection criteria were considered as true Endemic Normal (EN) individuals. At the end of the third year only 21 individuals remained who fulfilled all the criteria and were also willing to participate in the study. Out of these 21 individuals 6 were found to be suffering from other diseases and hence, had to be dropped out of the study. The remaining 15 asymptomatic microfilaremic individuals were included in the study as true endemic normal (EN) individuals.
### Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>NEN (n=15)</th>
<th>EN (n=15)</th>
<th>ASM (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of individuals included in the study</td>
<td>Males =10, Females=5, Total = 15</td>
<td>Males =8, Females=7, Total = 15</td>
<td>Males =7, Females=4, Total = 11</td>
</tr>
<tr>
<td>Age in years(mean ± sd) (range)</td>
<td>34.2±10.9 (19 - 56)</td>
<td>36.8±9.7 (21 - 53)</td>
<td>28.6±11.1 (16 - 48)</td>
</tr>
<tr>
<td>Presence of clinical symptoms of lymphatic filariasis</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Presence of Microfilaria in peripheral circulation at night</td>
<td>Absent</td>
<td>Absent</td>
<td>Yes Mf density 1–87/20μl</td>
</tr>
<tr>
<td>Level of CFA antigen as measured by Og4C3 ELISA* (mean ± sd)</td>
<td>16.7±4.35</td>
<td>15.0±4.41</td>
<td>6891.6 ± 4020.49</td>
</tr>
</tbody>
</table>

Table-2: Clinical and parasitological Status of the study groups.

*100units/ml of CFA antigen was taken as the cut-off.
Results

The non-endemic normal (NEN) individuals were selected from 5 villages around Leh, which was more than 10,000 ft above sea level, where transmission of filarial parasite has not been reported yet. 65 individuals were given a clinical examination to ascertain that they were free from any chronic or acute illness including lymphatic filariasis. 58 individuals were further tested for circulating microfilaria by taking finger prick blood between 22:00hrs and 2:00hrs., CFA by Og4C3 ELISA and parasite specific IgG4 titre by isotyping ELISA. All the 58 individuals tested negative in the above tests, but only 27 were willing to participate in the study and 15 individuals were selected out of them by age and sex matching to be included in the study as non-endemic normal (NEN) individuals (Flowchart-2).

**Og4C3 ELISA :**

The Circulatory Filarial Adult (CFA) antigen levels in the sera of NEN, EN and ASM individuals were measured by Og4C3 ELISA and the result shown in Fig.-2A. There was no significant difference in the mean values ($P=0.3952$) of CFA in NEN ($16.7 \pm 4.35$) and EN ($15.0 \pm 4.41$) individuals, while the ASM individuals had much higher values of CFA ($6891.6 \pm 4020.49$), which was significantly different than either NEN ($P<0.0001$) or EN ($P<0.0001$) individuals. Rank Sum test was used to compute the statistical significance between the mean of the different groups.
Results

Total Number of Individuals Screened from Leh
65 (age: 17-56yrs.)
(M: 27, F: 38)

Free of other Diseases
58 (age: 17-56yrs.)
(M: 25, F: 33)

Having other Diseases
7 (age: 34-53yrs.)
(M: 2, F: 5)

Detection of Microfilaria in Peripheral Circulation at night

All Negative

Og4C3 ELISA

All Negative

IgG4 titre to BmA

All Negative

Willing to participate in the study*

27 (age: 19-56yrs.)
(M: 12, F: 15)

Flowchart - 2: Screening of resident population of Leh for NEN individuals.

*
Results

Fig. 2: Circulatory Filarial Adult antigen (A) and B. malayi adult soluble antigen specific IgG4 titre (B) in the sera of NEN (n=15), EN (n=15) & ASM (n=11) individuals.
**Results**

**B. malayi Adult Soluble Antigen Specific IgG4 Titre:**

*B. malayi* adult soluble antigen specific IgG4 titre was determined by isotyping ELISA (Fig.-2B). It was found that the ASM individuals (8000.0 ± 2469.8) had a significantly ($P<0.0001$) higher titre of parasite specific IgG4 titre than either EN (350.0 ± 207.2) or NEN (28.9 ± 12.1) individuals. The difference between the values in EN and NEN individuals were also found to be significant ($P<0.0001$).

**Total Immunoglobulin Titre Against B. malayi L3 Soluble Antigen:**

The total immunoglobulin titre against *B. malayi* L3 soluble antigen was determined in NEN, EN and ASM individuals by ELISA (Fig.-3A). It was observed that the mean titre of the NEN (38.4 ± 11.9) individuals were significantly different than that of either EN ($P=0.007$) or ASM ($P=0.007$). The mean Ig titres against BmL3 in EN and ASM individuals were 1840.0 ± 806.6 and 5300.0 ± 930.1 respectively, which was significantly different ($P=0.03$). Thus it can be assumed from these results that the NEN individuals had not been exposed to BmL3 prior to inclusion in the study.

**Total Immunoglobulin Titre Against B. malayi Adult Soluble Antigen:**

The sera of all the NEN, EN and ASM individuals were assayed to determine the total immunoglobulin titre against BmA by ELISA. The actual titre values are shown in Fig.-3B. There was significant differences ($P<0.0001$) between the mean titre values of NEN.
Results

Fig. 3: Total immunoglobulin titre against *B. malayi* L3 soluble antigen (A) and adult soluble antigen (B) in the sera of NEN (n=15), EN (n=15) & ASM (n=11) individuals.
Results

(31.7 ± 13.7) and EN (3570.0 ± 1775.4) individuals, as well as between NEN and ASM (12772.7 ± 2649.2) individuals \( (P<0.0001) \). The difference between the EN and ASM individuals were also found to be significant \( (P<0.0001) \) by rank sum test.

**Antibody Response of NEN, EN and ASM Individuals to H37Rv Whole Cell Lysate (H37Rv-wcl):**

The total immunoglobulin titres against H37Rv-wcl (Fig.-4B) did not show any significant difference \( (P=0.06) \) between NEN (5740.0 ± 1767.9) and EN (4093.3 ± 1603.8) groups. The ASM group showed a slightly depressed antibody response (2663.6 ± 1372.8) against H37Rv-wcl, which appeared to be significantly different from both NEN \( (P = 0.01) \) and EN \( (P=0.03) \) individuals.

**Recognition of B.malayi Adult Soluble Antigens by IgG Antibodies in the sera of NEN, EN and ASM individuals by Immunoblot:**

It was observed that the ASM individuals in contrast to EN individuals recognized low molecular weight (14-20kDa) antigens of 3mA. The EN on the other hand recognized higher molecular weight antigens (data not shown). The NEN sera did not react with any parasite antigens, as no bands were visible even after prolonged incubation with the developing solution. All the ASM individuals consistently recognized the low molecular weight antigens of BmA. However, in case of EN no consistent pattern of recognition was observed, except that they recognized higher molecular weight antigens and the low molecular weight (14-20kDa) antigens were not seen.
Fig.-4: (A) IFN-γ to IL-4 ratio in response to *B. malayi* adult soluble antigen and (B) total immunoglobulin titre against H37Rv whole cell lysate in NEN (n=15), EN (n=15) & ASM (n=11) individuals.
Results

*creted IFN-γ to IL-4 Ratio in Response to B. malayi Adult Luble Antigen in vitro:*

PBMCs (10⁶/ml) of NEN, EN and ASM individuals were cultured *vitro* in a flat bottom 96 well tissue culture plate in the presence of optimal concentration (10μg/ml) of BmA and supernatants collected at 48hrs. for assaying secreted IL-4, and at 96hrs. for assaying secreted IFN-γ. The ratio of IFN-γ to IL-4 was obtained and plotted as a vertical scatter for each group of individuals (Fig.-4A). The mean ratio of secreted IFN-γ to IL-4 by PBMCs of EN individuals (1.8 ± 40.8) in response to BmA were significantly (P<0.0001) higher than that of either NEN (7.72 ± 5.4) or ASM (1.25 ± 0.6) individuals. The difference between the mean values of NEN and ASM individuals were also found to be significantly (P<0.0001) different. The observed differences are mostly due to a skewing of response in the EN and ASM groups. The EN individuals tend to secrete more IFN-γ than IL-4, while it is just reversed in the case of ASM individuals. The values for NEN individuals being in between these groups. The clinical, parasitological and immunological profile of the three study groups have been summarized in Tables-3.
<table>
<thead>
<tr>
<th></th>
<th>NEN (n=15)</th>
<th>EN (n=15)</th>
<th>ASM (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG4 titre against <em>B. malayi</em> adult soluble antigen (mean±s.d.)</td>
<td>25.0 ± 12.1</td>
<td>350.0 ± 207.2</td>
<td>8000.0 ± 2469.8</td>
</tr>
<tr>
<td>Ig titre against <em>B. malayi</em> L3 soluble antigen (mean±s.d.)</td>
<td>35.0 ± 11.9</td>
<td>1840.0 ± 806.6</td>
<td>5300.0 ± 930.1</td>
</tr>
<tr>
<td>Ig titre against <em>B. malayi</em> adult soluble antigen (mean±s.d.)</td>
<td>30.0 ± 13.7</td>
<td>3570.0 ± 1775.4</td>
<td>12700.0 ± 2649.2</td>
</tr>
<tr>
<td>Presence of IgG antibodies specific to low molecular weight (14-20kDa) proteins of <em>B. malayi</em> adult soluble antigen as visualized by immunoblot</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>In vitro</em> secreted IFN-γ to IL-4 ratio in response to <em>B. malayi</em> adult soluble antigen (mean±s.d.)</td>
<td>7.72 ± 5.4</td>
<td>63.8 ± 40.8</td>
<td>1.25 ± 0.66</td>
</tr>
</tbody>
</table>

**Table-3:** Immunological profile of the study groups as determined using *B. malayi* adult and L3 soluble antigens.
Results

ANTIGENS:

For the various immunological studies we have used somatic antigens from *B. malayi* adult (BmA) and L3 (BmL3) parasites. The other antigen used in this study was the prototype *Mycobacterium tuberculosis* strain – H37Rv whole cell lysate.

The SDS-PAGE (12%) profile of all these antigens are shown in Fig.-5. The profile of H37Rv-wcl was found to be different as expected from both *B. malayi* L3 and *B. malayi* adult soluble antigen. There was significant differences between L3 and adult soluble antigens also. In Coomassie blue staining at least 53 bands were visible in the adult soluble antigen while only 37 bands were visible for the L3 soluble. The L3 soluble had a few prominent bands in the higher molecular weight range, between ~200 – 95kDa, which were not visible in the adult soluble antigen. The adult soluble antigen showed prominent bands between ~25 – 12kDa which were not very clearly visible in the L3 soluble antigen. Since proteolytic enzyme inhibitors like TLCK, TPCK and PMSF were used during preparation of the antigens, the lower molecular weight bands are probably not due to proteolytic degradation of higher molecular weight bands.
**Results**

**Fig.-5:** Protein profile of antigens visualized on a 12% SDS-polyacrilamide gel after staining with Coomassie brilliant blue (R-250)

Lane a: *B. malayi* L3 soluble antigen  
Lane b: *B. malayi* Adult soluble antigen  
Lane c: H37Rv whole cell lysate  
Lane d: Molecular weight marker
OPTIMAL ANTIGEN CONCENTRATIONS REQUIRED FOR STIMULATION OF PERIPHERAL BLOOD LYMPHOCYTES:

To determine the optimum concentration of *B. malayi* adult soluble antigen (BmA), live adult, live L3 and H37Rv whole cell lysate (H37Rv-wcl), peripheral blood mononuclear cells (PBMCs) from 4 NEN and 4 EN individuals were taken and stimulated with different concentrations of the antigens. PBMCs were cultured along with 2.5, 5.0, 10.0, 15.0 or 20.0μg/ml of the soluble antigens viz. BmA, or H37Rv-wcl (irrelevant antigen). For live L3 and live Adult, PBMCs were cultured in the presence of live parasites. Live L3 were cultured at 10, 20, 30, 40 or 50 live parasites/ml along with cells at a concentration of 10⁶/ml. Similarly live Adult parasites were put at 1, 2, 3, 4 and 5 parasites per well. Stimulation indices (SI) were calculated and the minimum antigen concentration giving the maximum SI was taken as the optimum concentration for that antigen. Both BmA and H37Rv-wcl were found to stimulate human PBMCs optimally at 10.0μg/ml concentration (Fig.-6A & 7A). 20 parasites/ml in case of live L3 (Fig.-6B) and 2 parasites/ml in case of live adult (Fig.-7B) were found to be best suited for human PBMC stimulation. Thereafter throughout the study PBMCs from NEN, EN and ASM individuals were cultured using the optimum concentrations of the respective antigens and optimal number of the respective parasites.
Fig.-6: Determination of the optimum H37Rv whole cell lysate concentration (A) and optimum numbers of *B. malayi* live L3 (B) required to stimulate PBMCs from NEN (n=4) and EN (n=4) individuals.
Fig. 7: Determination of Optimum *B. malayi* adult soluble antigen concentration (A) and optimum number of adult parasites (B) required for the stimulation of PBMCs from NEN (n=4) & EN (n=4) individuals
DIFFERENTIAL PROLIFERATION OF PBMCs OF NEN, EN & ASM INDIVIDUALS IN RESPONSE TO DIFFERENT ANTIGENS:

Generally it was found that the response of the ASM individuals to all the antigens were suppressed when compared to that of NEN and EN individuals. With H37Rv-wcl there was no significant differences between the mean stimulation index (SI) values of NEN (14.44 ± 2.78) and EN (13.09 ± 3.32) individuals (P=0.1585). But when compared to ASM (9.93 ± 3.03) the differences with either NEN or EN were found to be significant, P=0.0026 and P=0.0240 respectively (Fig.8A).

Among the three different groups *B. malayi* adult soluble antigen was able to stimulate EN most (Fig.-8C). The mean SI value of EN (6.9 ± 1.51) group was significantly higher than both NEN (P<0.0001) and ASM (P=0.0001). However, between NEN and ASM individuals, it was the NEN group which showed a higher (4.50 ± 0.84) SI than the ASM group (3.13 ± 0.82). Rank sum test found the differences between the SI of NEN and ASM to be significant (P<0.0001). But, *B. malayi* live adult (Fig.-8D) induced comparable amounts of cell proliferation in both NEN and ASM individuals (P=0.1711). The ASM individuals response was poor (2.31 ± 0.63) to live adult parasite and was significantly lower than that of NEN (P<0.0001) and EN (P<0.0001).

*B. malayi* live L3 induced maximal proliferation (SI=6.33 ± 0.95) in the EN group (Fig.-8B), which was found to be significantly higher than both NEN (SI=5.17 ± 1.04, P=0.0090), and ASM (SI=3.18 ±
Results

0.84, $P<0.0001$). The differences between the SI of NEN and ASM was also found to be significant ($P<0.0001$). The mean SI value ± standard deviation along with range against all the different antigens used, has been tabulated for all the groups, in Table-4.
Results

Fig. 8: Stimulation Indices of PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to H37Rv whole cell lysate (A), *B. malayi* live L3 (B), adult soluble antigen (C) and live adult parasites (D). Antigens and live parasites were used at optimal concentration and numbers respectively.
### Results

#### Optimum Concentration of Stimulation Index

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Optimum Concentration of antigen or no. of parasites</th>
<th>Stimulation Index Mean ± s.d. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NEN (n=15)</td>
</tr>
<tr>
<td>H37Rv whole cell Lysate</td>
<td>10 µg/ml</td>
<td>14.44 ± 2.78 (9.7-19.4)</td>
</tr>
<tr>
<td>B. malayi live L3</td>
<td>20 parasites/ml</td>
<td>5.17 ± 1.04 (3.4-6.9)</td>
</tr>
<tr>
<td>B. malayi adult soluble antigen</td>
<td>10 µg/ml</td>
<td>4.50 ± 0.84 (2.8-5.6)</td>
</tr>
<tr>
<td>B. malayi live adult adult</td>
<td>2 parasites/ml</td>
<td>5.10 ± 1.06 (3.0-6.8)</td>
</tr>
</tbody>
</table>

Table-4: Stimulation indices determined by stimulation of PBMCs taken from NEN (n=15), EN (n=15) & ASM (n=11) individuals with optimal antigen concentrations and live parasite numbers respectively.
THE RELATIVE NUMBER OF CD3+, CD4+ AND CD8+ CELLS IN NEN, EN AND ASM INDIVIDUALS AFTER STIMULATION WITH THE DIFFERENT ANTIGENS:

Relative frequencies of CD3+, CD4+ and CD8+ cells were determined by flowcytometry, 96hrs. after stimulation with the different antigens. We had stained cells stimulated with the optimal concentration of antigens and numbers of parasite at different time points starting from 24 to 96hrs., and found that the maximal proliferation occurs at 96hrs. Individuals belonging to all the three groups showed upregulation of CD3+ cells, though ASM group showed the least. H37Rv-wcl was able to induce maximum proliferation of CD3+ cells in all the three groups. None of the filarial antigens were able to induce appreciable proliferation of these cell types in ASM individuals. EN individuals responded best to live L3 parasite, among all the different filarial antigens (Fig.-10). The CD4+population also showed the same profile, with the EN showing the maximum proliferation against the filarial antigens, while the ASM individuals showed the minimum (Fig.-11). However, both EN and NEN showed upregulation of CD8+ population in response to BmA, live adult and live L3 parasites, while the ASM individuals did not show much (Fig.-12).
Results

Fig. -9: Forward vs. Side scattergram obtained upon flowcytometric analysis of unstained human PBMC population.

R1 Lymphocytes
R2 Monocytes
R3 Granulocytes
R4 Dead cells
Fig. - 10: Representative data of flowcytometric analysis of CD3+ lymphocyte population in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Results

Fig. 11: Representative data of flowcytometric analysis of CD4+ lymphocyte population in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Fig. -12: Representative data of flowcytometric analysis of CD8+ lymphocyte population in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Results

Fig. -13: Representative data of flowcytometric analysis of HLA-DR, DP & DQ expressing cell population in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Results

Fig. 14: Representative data of flow cytometric analysis of CD4+ lymphocyte population expressing HLA-DR in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
EXPRESSION OF HLA – DR, DP AND DQ BY PBMCs OF NEN, EN AND ASM INDIVIDUALS WHEN STIMULATED WITH OPTIMAL CONCENTRATIONS OF DIFFERENT ANTIGENS:

No appreciable differences could be noticed in the levels of expression of HLA – DR, DP and DQ on the lymphocytes and monocytes of the three groups of individuals, when stimulated with different antigens used in the study (Fig-13). However, differences in the level of expression of HLA-DR were observed on CD4+ cells in the three groups in response to different antigens. In response to BmA, live adult and live L3, the EN individuals had relatively more number of CD4+ cells expressing HLA-DR than either NEN or ASM individuals (Fig.-14).

KINETICS OF SECRETION OF IL-2, IL-4, IL-10, IL-12, IL-1β AND IFN-γ IN RESPONSE TO DIFFERENT ANTIGENS IN EN INDIVIDUALS:

PBMCs from all the 15 EN individuals were cultured in the presence or absence of the optimal concentrations of the different antigens, viz. H37Rv-wcl, BmA, live adult and live L3 parasites. Supernatants were collected after 24hrs., 48hrs., 72hrs., and 96hrs. of culture. The supernatants were assayed for quantitation of IL-2, IL-4, IL-10, IL-12, IL-1β and IFN-γ. Fig.-15 &16 show the kinetics of secretion of the above-mentioned cytokines by PBMCs of EN individuals in response to the different antigens used in this study. It was observed that in response to BmA the PBMCs of EN individuals'
Fig. - 15: Kinetics of secretion of IL-2 (A), IL-4 (B) and IL-10 (C) by PBMCs of EN (n=15) individuals in response to *B. malayi* adult soluble antigen
Fig. -16: Kinetics of secretion of IL-12 (A) and IL-1β (B) and IFN-γ (C) by PBMCs of EN (n=15) individuals in response to *B. malayi* adult soluble antigen.
Results

peak secretion time for IL-2 was 72hrs., for IL-4 48hrs., for IL-10 24hrs., for IL-12 48hrs., for IL-1β 24hrs., and for IFN-γ 96hrs.

Similarly kinetics of secretion of all these cytokines in response to all the antigens were determined for NEN and ASM individuals (data not shown). From the kinetics, the peak time point of secretion of a particular cytokine against a definite antigen was determined for NEN, EN and ASM. Throughout the study all cytokine values reported are the peak secretion level of that cytokine for a particular group in response to a particular antigen.

DIFFERENTIAL SECRETION OF IL-2 BY PBMCs OF NEN, EN & ASM INDIVIDUALS IN RESPONSE TO THE DIFFERENT ANTIGENS:

Figure-17 shows the levels of IL-2 secreted by the PBMCs of NEN, EN and ASM individuals when kept without any antigen. No significant differences in the levels of secretion of IL-2 between the groups were observed under this condition.

Both B. malayi adult soluble antigen and live adult (Fig.-18) induced maximum IL-2 in EN group and the least in the ASM group. BmA induced significantly different levels of IL-2 between EN (249.3 ± 64.3pg/ml) and NEN (165.3 ± 48.1pg/ml, P<0.0001) and between EN and ASM (107.1 ± 48.2pg/ml, P<0.0001). NEN also secreted significantly more (P=0.0075) amounts of IL-2 than ASM in
Results

Fig.-17: Peak IL-2 secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals when cultured without any antigen.

NEN : EN - P=0.9835
NEN : ASM - P=0.2993
EN : ASM - P=0.2757
Fig. 18: Peak IL-2 secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to H37Rv whole cell lysate (A), B. malayi live L3 (B), adult soluble antigen (C) and live adult parasites (D).
### Table-5: Peak in vitro secretion of IL-2 by the PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to the optimal concentrations of antigens and numbers of live parasites respectively.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Optimum Concentration of antigen or no. of parasites</th>
<th>Peak IL-2 Secretion Mean ± s.d. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H37Rv whole cell Lysate</td>
<td>10 μg/ml (n=15)</td>
<td>NEN 470.1 ± 178.8 (203-786) EN 396.9 ± 96.2 (268-596) ASM 230.0 ± 54.0 (122-291)</td>
</tr>
<tr>
<td>B. malayi live L3</td>
<td>20 parasites/ml (n=15)</td>
<td>NEN 197.3 ± 38.2 (143-274) EN 267.3 ± 113.5 (79-497) ASM 150.9 ± 35.0 (99-197)</td>
</tr>
<tr>
<td>B. malayi adult soluble antigen</td>
<td>10 μg/ml (n=15)</td>
<td>NEN 165.3 ± 48.1 (92-261) EN 249.3 ± 64.3 (162-398) ASM 107.1 ± 48.2 (59-196)</td>
</tr>
<tr>
<td>B. malayi live adult</td>
<td>2 parasites/ml (n=15)</td>
<td>NEN 232.9 ± 51.3 (111-318) EN 302.1 ± 122.9 (129-514) ASM 88.3 ± 29.1 (53-134)</td>
</tr>
</tbody>
</table>

*Results*
response to BmA. Profile of IL-2 induction by live adult parasite was similar to BmA, except for the fact that the IL-2 secretion in ASM individuals was further suppressed (88.3 ± 29.1 pg/ml), when compared to BmA. Live L3 (Fig.-18) induced slightly higher levels of IL-2 in EN (267.3 ± 113.5 pg/ml) than in NEN (197.3 ± 38.2 pg/ml, P=0.0401). But ASM individuals secreted less quantities of IL-2 (150.9 ± 35.0 pg/ml) in response to L3.

In case of H37Rv whole cell lysate ASM (230.0 ± 54.0 pg/ml) secreted less amounts of IL-2 than either NEN (470.1 ± 178.8 pg/ml) or EN (396.9 ± 96.2 pg/ml). NEN group secreted slightly more amounts of IL-2 than EN group, though the difference was not significant (P=0.2290). The mean quantities of IL-2 secreted by the different groups in response to the above mentioned antigens are tabulated in Table-5.

**RELATIVE NUMBER OF CELLS EXPRESSING IL-2 RECEPTORS ON THEIR SURFACE BY FLOWCYTOMETRY:**

IL-2 receptor α (IL-2Rα) expression was found to peak at 48hrs. and the NEN individuals have been found to express the maximum amounts of IL-2Rα when compared to its no antigen control (Fig.-19). Both BmA and live adult induced comparable amounts of IL-2Rα, while L3 induced slightly less, which was not significant. Both EN and ASM individuals expressed little IL-2Rα in response to filarial antigens. However, EN expressed more IL-2Rα than the ASM, which was found to be significant (P<0.0001).
Fig.-19: Representative data of flowcytometric analysis of lymphocyte population expressing IL-2 receptor-α in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
EXPRESSION OF CD69 ON THE SURFACE OF LYMPHOCYTES FROM NEN, EN AND ASM INDIVIDUALS IN RESPONSE TO DIFFERENT ANTIGENS.

Expression of CD69 on the surface of the lymphocytes of NEN, EN and ASM were seen after 24hrs of culture with the different antigens. A pilot kinetic study showed that the expression of CD69 molecule on the surface of human lymphocytes in response to the filarial as well as H37Rv-wcl peaks at 24hrs. The results show that in response to H37Rv-wcl lymphocytes of NEN, EN and ASM individuals expressed comparable amounts of CD69 molecule on their surface (Fig.-20). The ASM individuals expressed minimum levels of CD69 on their lymphocyte surface in response to B.malayi live adult and soluble adult antigens. In contrast live L3 induced higher levels of expression of CD69 on the lymphocytes of ASM individuals. The NEN and EN individuals expressed similar levels of CD69 on their lymphocyte surface in response to live adult and adult soluble antigens. But, in response to live L3 the lymphocytes of EN individuals expressed significantly higher levels of CD69 than that of NEN.
Results

Fig.-20: Representative data of flowcytometric analysis of lymphocyte population expressing CD69 in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Results

RELATIVE FREQUENCIES OF CD45RO+ CD4+ CELLS IN NEN, EN AND ASM INDIVIDUALS AFTER IN VITRO CULTURING OF THEIR PBMCs WITH DIFFERENT ANTIGENS:

The relative numbers of CD45RO+ CD4+ cells were determined after 96hrs. of culture with the different antigens used in this study. We had stained cells stimulated with the optimal concentration of antigens and numbers of parasite at different time points starting from 24 to 96hrs., and found that the maximal proliferation occurs at 96hrs. It was observed that H37Rv whole cell lysate induced comparable levels of expression of the phenotype in all the three groups of individuals. Interestingly, not many CD45RO+ CD4+ cells were induced in ASM individuals in response to BmA, live adult and live L3 (Fig.-21). EN individuals in contrast, expressed a significantly more number of the phenotype in response to live L3 parasites. However, in response to the live adult parasite or BmA expansion of CD45RO+ CD4+ cells in EN individuals were not as high as seen with live L3. Slight increase in the phenotype was observed in the case of NEN individuals in response to BmA, live adult and live L3, which was not found to be significant.
Fig.-21: Representative data of flowcytometric analysis of CD4+ lymphocyte population expressing CD45RO in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
LEVELS OF IL-1β SECRETED BY PBMCs OF NEN, EN & ASM INDIVIDUALS IN RESPONSE TO DIFFERENT ANTIGENS.

We could not detect any significant difference in the peak secretion of IL-1β between the different groups when stimulated with H37Rv whole cell lysate or live L3 (Fig.-23A). However, *B. malayi* live adult parasite (Fig.-23D) was able to induce high levels of IL-1β in ASM individuals (533.1 ± 165.3), which was significantly higher than that of EN (*P*=0.0147) but not that of NEN (*P*=0.6404). The mean quantities of IL-1β secreted by the three different groups in response to the different antigens are tabulated in Table-6. There was no significant differences between the levels of secreted IL-1β by PBMCs of NEN, EN and ASM individuals when cultured without any antigen (Fig.-22).

DIFFERENTIAL INDUCTION OF IFN-γ AND IL-4 IN NEN, EN AND ASM INDIVIDUALS BY DIFFERENT ANTIGENS.

Peak secretion of IFN-γ and IL-4 levels were compared in all the three groups. It was observed that H37Rv-wcl induced the maximum levels of IFN-γ in all the three groups (Fig.-25A), however the ASM individuals secreted comparatively lesser amounts of IFN-γ (939.5 ± 359.7pg/ml) compared to that of NEN (4013.7 ± 1869.9pg/ml) and EN (3347.5 ± 1742.3pg/ml).
Results

individuals. The difference in the secretion is not significant between NEN and EN ($P=0.9669$), but is

![Graph showing IL-1β secretion in NEN, EN, and ASM individuals](image)

**Fig.-22:** Peak IL-1β secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals when cultured without any antigen.
Fig.-23: Peak IL-1β secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to H37Rv whole cell lysate (A), B. malayi live L3 (B), adult soluble antigen (C) and live adult parasites (D)
### Table-6: Peak in vitro secretion of IL-1β by the PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to the optimal concentrations of antigens and numbers of live parasites respectively.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Optimum Concentration of antigen or no. of parasites</th>
<th>Peak IL-1β Mean ± s.d. (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H37Rv whole cell Lysate</td>
<td>10 μg/ml</td>
<td>NEN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>593.1 ± 163.7 (331-740)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500.4 ± 79.8 (405-638)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASM (n=11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>543.5 ± 156.3 (245-789)</td>
</tr>
<tr>
<td><em>B. malayi</em> live L3</td>
<td>20 parasites/ml</td>
<td>NEN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>350.3 ± 168.5 (147-653)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>229.1 ± 104.6 (123-490)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASM (n=11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>274.1 ± 138.3 (94-528)</td>
</tr>
<tr>
<td><em>B. malayi</em> adult soluble antigen</td>
<td>10 μg/ml</td>
<td>NEN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>285.4 ± 99.2 (95-645)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>172.1 ± 30.2 (140-236)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASM (n=11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>407.6 ± 156.0 (169-563)</td>
</tr>
<tr>
<td><em>B. malayi</em> live adult</td>
<td>2 parasites/ml</td>
<td>NEN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>464.5 ± 201.8 (121-712)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EN (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>324.6 ± 208.0 (116-693)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ASM (n=11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>533.1 ± 165.3 (313-824)</td>
</tr>
</tbody>
</table>

Results
Results

significant between ASM and NEN ($P=0.0022$) as well as between ASM and EN ($P<0.0001$). BmA, live adult and live L3 induced comparatively more of IFN-$\gamma$ in EN individuals than in ASM (Fig.26, 27, & 28), in all the cases the difference is significant ($P<0.0001$). Between live adult and live L3, the latter ($234.4 \pm 112.3\text{pg/ml}$) was able to induce more of IFN-$\gamma$ secretion in ASM individuals than either live adult ($90.9 \pm 53.1\text{pg/ml}$) or BmA ($203.5 \pm 100.4\text{pg/ml}$). However the difference between the amount of IFN-$\gamma$ secreted by ASM individuals in response to BmA and live L3 were not significant ($P=0.1341$).

IL-4 secretion was maximally induced by live adult of *B. malayi* in NEN ($40.7 \pm 23.6\text{pg/ml}$) and EN ($104.7 \pm 34.6\text{pg/ml}$) groups (Fig.-28A) and by BmA (Fig.-27A) in the ASM group ($160.3 \pm 51.3\text{pg/ml}$). H37Rv-wcl induced very little or no IL-4 in all the three groups (Fig.-25). It is interesting to note that in the EN individuals BmA was not able to induce much of IL-4 secretion ($21.2 \pm 10.3\text{pg/ml}$), but live adult was. The difference in these secretion levels were also significant ($P<0.0001$). It appears that the live adult is able to induce a preferentially Th2 kind of response in the EN individuals. To understand the skewing of response induced by these antigens clearer we plotted the IFN-$\gamma$ vs. IL-4 values in each individual as a scatter (Fig.-25 to 28). No clear polarization was observed between the three groups in case of H37Rv-wcl, though the ASM individuals appear to segregate as they produced lesser amounts of IFN-$\gamma$. But the response against BmA was highly polarized between EN and ASM individuals. The EN predominantly mounted a Th1 response while the ASM mounted predominantly a Th2 response, while the NEN individuals were
Results

Fig.-24: IFN-γ vs. IL-4 (A) and IL-12 vs. IL-10 (B) secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals when cultured without any antigen.
Fig.-25: IFN-γ vs. IL-4 (A) and IL-12 vs. IL-10 (B) secreted by PBMCS of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to H37Rv whole cell lysate.
Fig. 26: IFN-γ vs. IL-4 (A) and IL-12 vs. IL-10 secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to *B. malayi* live L3 parasite
somewhat in between these two groups. Live adult on the other hand induced sufficient quantity of IL-4 in EN individuals without significantly affecting their ability to secrete IFN-γ. Similarly live L3 was able to induce higher amounts of IFN-γ in ASM individuals.

DIFFERENTIAL INDUCTION OF IL-10 AND IL-12 IN NEN. EN AND ASM INDIVIDUALS BY DIFFERENT ANTIGENS:

Comparison of the level of IL-10 secreted by the PBMCs of individuals from the different study groups revealed that H37Rv-wcl induces very high levels of the cytokine at 24hrs in all the groups. The ASM individuals (Fig.-25B) produced the maximum levels (931.5 ± 362.7pg/ml), which however, was not significantly different from either NEN (583.9 ± 364.2pg/ml, $P=0.3776$) or EN (373.9 ± 219.8pg/ml, $P=0.0516$). It is interesting to note that the PBMCs of ASM (Fig.-24B) individuals secreted a much higher quantity of IL-10 without any antigen (296.4 ± 106.1pg/ml) than NEN (60.8 ± 19.3pg/ml) or EN (70.1 ± 29.4pg/ml). BmA, live adult as well as live L3 induced very high levels of IL-10 in ASM individuals, while the EN individuals secreted minimal amounts of IL-10 in response to these antigens (Fig.-26 to 28). NEN also secreted high amounts of IL-10 in response to live adult (508.3 ± 231.2pg/ml) and live L3 (561.7 ± 196.8pg/ml) but not in response to BmA (40.3 ± 16.8pg/ml). Both live adult (119.8 ± 46.7pg/ml) and live L3 (108.9 ± 41.3pg/ml) was able to induce high levels of IL-12 in NEN individuals, but BmA (85.6 ± 29.1pg/ml) induced comparatively lesser amounts. The ASM individuals secreted the minimum amounts of IL-12 in response to
Results

Fig.-27: IFN-γ vs. IL-4 (A) and IL-12 vs. IL-10 (B) secreted by PBMCs of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to B. malayi adult soluble antigen.
Results

Fig.-28: IFN-γ vs. IL-4 (A) and IL-12 vs. IL-10 (B) secreted by PBMCS of NEN (n=15), EN (n=15) & ASM (n=11) individuals in response to *B.malayi* live adult parasite.
the parasite antigens. IL-10 vs. IL-12 scatter plots are obtained to look into the polarization of these two counter regulatory cytokines in different groups in response to the different antigens used in this study.

**RELATIVE NUMBERS OF IFN-γ, IL-4 AND IL-10 PRODUCING CELLS IN NEN, EN AND ASM AFTER STIMULATION WITH DIFFERENT ANTIGENS BY FLOWCYTOMETRY**:

PBMCs from NEN, EN and ASM individuals (3 in each group) were cultured along with H37Rv-wcl, BmA, AsA, live adult and live L3. Cells were harvested at 24hrs. for IL-10, 48hrs. for IL-4 and 96hrs. for IFN-γ. To determine these time points staining was done in a pilot experiment at all the 4 time points for all the three cytokines. Fig.30 to 32 shows the results which is representative of three different individuals in each group. Both EN and NEN individuals upregulated IFN-γ secreting cells upon stimulation with BmA and L3, which were significantly more than that expressed by ASM individuals (Fig.-30). In response to H37Rv-wcl all the groups had enhanced numbers of IFN-γ secreting cells, though ASM individuals had comparatively lesser number of cells. In case of IL-4 the scenario was just reversed, the ASM individuals upregulated IL-4 secreting cells in response to BmA, live adult and live L3 (Fig-31). However, live adult was able to increase the number of IL-4 secreting cells in both EN and NEN as well, while BmA and live L3 could not. H37Rv-wcl induced IL-10 secreting cells in all the groups of individuals to a (Fig.-32)
Fig. -29: Overlay histograms of isotype control, recombinant cytokine neutralized control, and positive control of internal cytokine staining for IFN-γ (A), IL-10 (B), and IL-4 (C)
Fig. - 30: Representative data of flowcytometric analysis of lymphocyte population producing IFN-γ in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Fig.-31: Representative data of flowcytometric analysis of lymphocyte population producing IL-10 in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Fig.-32: Representative data of flowcytometric analysis of lymphocyte population producing IL-4 in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
comparable degree. However, there was no increase in the number of IL-10 secreting cells in EN when stimulated with either BmA, or live adult, or live L3. The ASM individuals on the other hand expressed very high numbers of IL-10 secreting cells in response to these antigens. It is interesting to note that the NEN group had also increased numbers of IL-10 secreting cells when stimulated with parasite antigen or the live parasites.

To show that the internal cytokine staining were specific, cells were stimulated with PMA and ionomycin and stained with labelled isotype control, antibody blocking control and the labelled anti-cytokine antibody (Fig.-29).

**RELATIVE NUMBER OF CELLS EXPRESSING RECEPTORS FOR IL-4, IL-10 AND IL-12 ON THEIR SURFACE BY FLOWCYTOMETRY:**

IL-4 receptor (IL-4R) was also assayed at 48hrs. post culture and it was found that neither NEN nor EN expressed much of IL-4R in response to any of the filarial antigens when compared to their respective no antigen controls, though EN individuals expressed IL-4R in response to live adults. The ASM individuals expressed very high levels of IL-4R in response to BmA and live adult. The expression level was much less with live L3 (Fig.-33).

IL-10 receptor (IL-10R) was found to peak at 24 hrs. and the ASM individuals were found to express the maximum amounts of IL-10R
Fig.-33: Representative data of flowcytometric analysis of lymphocyte population expressing IL-4 receptor in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Fig. 34: Representative data of flowcytometric analysis of lymphocyte population expressing IL-10 receptor in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Fig.-35: Representative data of flowcytometric analysis of lymphocyte population expressing IL-12 receptor in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Results

in response to filarial antigens, while the EN expressed the minimum. The NEN individuals also expressed appreciable quantities of IL-10R on their lymphocyte surfaces. The ASM individuals expressed comparable amounts of IL-10R in response to BmA, live adult and live L3 parasites (Fig.-34).

When IL-12 receptor (IL-12R) was assayed on the surface of lymphocytes and monocytes, it was found that the EN individuals expressed very high IL-12R on stimulation with BmA, live adult and live L3, while the ASM individuals expressed very little IL-12R when compared with their respective no antigen controls. The NEN individuals expressed moderate amounts of IL-12R in response to the parasite antigens. Individuals of all the three groups expressed appreciable amounts of IL-12R on stimulation with H37Rv-wcl (Fig.-35).

EXPRESSION OF CD80 AND CD86 ON THE MONOCYTES OF NEN, EN AND ASM INDIVIDUALS IN RESPONSE TO THE DIFFERENT ANTIGENS:

It was observed that both CD80 and CD86 expressions were downregulated by H37Rv-wcl antigen. BmA, live adult and live L3 were found to slightly upregulate the expression of CD80 (Fig.-36) and CD86 (Fig.-37) in EN and NEN individuals. However, the same filarial antigens appeared to down regulate the expression of CD80 and CD86 on the monocytes of ASM individuals.
Fig. 36: Representative data of flowcytometric analysis of monocytes expressing CD80 in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Results

Fig.-37: Representative data of flowcytometric analysis of monocytes expressing CD86 in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
EXPRESSION OF CD40 AND CD40L ON THE PBMCs OF NEN, EN AND ASM INDIVIDUALS IN RESPONSE TO THE DIFFERENT ANTIGENS USED IN THIS STUDY:

Expressions of CD40 and CD40L were determined on the lymphocytes of NEN, EN and ASM in response to H37Rv whole cell lysate, *B. malayi* adult soluble antigen, live adult and live L3. We did not observe any appreciable difference in the levels of expression of either CD40 or CD40L in the three groups in response to any of the antigens used (Fig. 38 & 39).
Fig.-38: Representative data of flowcytometric analysis of lymphocytes expressing CD40 in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.
Fig.-39: Representative data of flowcytometric analysis of lymphocytes expressing CD40L (CD154) in NEN (n=3), EN (n=3) and ASM (n=3) individuals in response to different antigens. K-S statistics was applied and the values given.