CHAPTER - 5

PEPTIDE AS INHIBITORS OF HUMAN COMPLEMENT ACTIVATION
5. INTRODUCTION

The pivotal role of complement mediated injury in a wide variety of disease such as arthritits,\textsuperscript{238} respiratory diseases,\textsuperscript{239} heart attacks,\textsuperscript{240} burn injuries,\textsuperscript{241} etc has been well established. The complement component C3 plays a central role in the complement activation process, which itself gets cleaved by C3 convertase into an anaphylotoxic peptide C3a and an opsonic fragment C3b.\textsuperscript{242} Binding of C3b to target cells undergoing complement attack results in generation of C5a and formation of C5-9 membrane attack complex, thereby resulting in host cell damage. Attempts to suppress complement activation have led investigators to identify a suitable complement inhibitor which can be therapeutically used in disease involving complement mediated damage. In this direction a variety of synthetic peptides or chemical compounds\textsuperscript{245-247} have been investigated for either targeting serine proteases serine proteases or complement component C3. Recently a soluble form of complement receptor 1 has been found to suppress complement in several complement dependent disease models.\textsuperscript{248} However, smaller fragment that can functionally mimic these proteins will have a better clinical acceptance than large poly peptides.

In our laboratory we have initiated screening a variety of structurally diverse synthetic peptides in order to identify the peptide ligand that inhibit C3 activation.\textsuperscript{249} In this chapter inhibition of human complement activation by novel hexapeptides related to IgE Fc region (330-334) is described. Some of these hexa peptides exhibited high order of antiallergic activity when administered both by i.p. as well as p.o. routes in rats reported in chapter 2 and 4 of this thesis.
5.1 Bioevaluation

In the first instance some of the most potent compound of antiallergic/antiallergic series were evaluated in hemolytic assay for their ability to inhibit classical pathway activity. All the novel hexapeptides discussed in this chapter were bioevaluated for inhibiting human complement activation in the Division of Biochemistry at Central Drug Research Institute, Lucknow.

The hemolytic complement assay\(^{250}\) was carried out using veronal saline buffer (25 mM) pH 7.3, containing 0.15 mM Ca\(^{++}\) and 0.5 mM Mg\(^{++}\) as diluent with fresh pooled human serum as a source of complement sensitized sheep erythrocytes were incubated with complement incubated peptides. The degree of hemolysis was determined spectrophotometrically at 413 nM. The results have been summarized in table 1.

5.2 Results and Discussion

As evident from table 1 peptides XVI, XXI and XXIV exhibited total inhibition of human complement whereas, peptides V, VII, VIII, X and XVII exhibited moderate to marked inhibition of human complement at 200 \(\mu\)g. It is interesting to note that peptides V, VII, VIII and X have the same c-terminal pentapeptide fragment (Asp-Ser-Asp-Gly-Lys) and the activity profile of compounds derived from this pentapeptide was found to vary with the modification in the N-terminal residue. On the otherhand significant inhibition exhibited by peptides XVI, XXI and XXIV had \(X_1\)-Gly-Gly-Asp-\(X_5\)-Lys as motif (\(X_1\) = D-Ala or Ala; \(X_2\) D-Ala or Sar). Recently Sahu et al\(^{251}\) have reported a 13 amino acid cyclic peptide which was found to be the most potent inhibitor of C3.
Table 1: Effect of peptides on the classical pathway of human complement activation (*in vitro*)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Peptide Sequence</th>
<th>% Inhibition 200 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Pro-Asp-Ser-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>II</td>
<td>Glp-Asp-Ser-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>III</td>
<td>AcAla-Asp-Ser-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>IV</td>
<td>D-Arg-Asp-Ser-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>V</td>
<td>Leu-Asp-Ser-Asp-Gly-Lys</td>
<td>77</td>
</tr>
<tr>
<td>VI</td>
<td>Val-Asp-Ser-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>VII</td>
<td>Glu-Asp-Ser-Asp-Gly-Lys</td>
<td>74</td>
</tr>
<tr>
<td>VIII</td>
<td>Ser-Asp-Ser-Asp-Gly-Lys</td>
<td>80</td>
</tr>
<tr>
<td>IX</td>
<td>Tyr-Asp-Ser-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>X</td>
<td>Arg-Asp-Ser-Asp-Gly-Lys</td>
<td>54</td>
</tr>
<tr>
<td>XIII</td>
<td>Ala-Gly-D-Ala-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XVI</td>
<td>Ala-Gly-Gly-Asp-D-Ala-Lys</td>
<td>100</td>
</tr>
<tr>
<td>XVII</td>
<td>Ala-Gly-Gly-Asp-Sar-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XVIII</td>
<td>Ala-D-Ala-Gly-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XX</td>
<td>Ala-Gly-Sar-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XXI</td>
<td>Ala-Gly-Gly-Asp-Sar-Lys</td>
<td>100</td>
</tr>
<tr>
<td>XXII</td>
<td>Ala-Gly-Sar-Asp-Sar-Lys</td>
<td>80</td>
</tr>
<tr>
<td>XXIII</td>
<td>Ala-Gly-Sar-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XXIV</td>
<td>D-Ala-Gly-Gly-Asp-Sar-Lys</td>
<td>100</td>
</tr>
<tr>
<td>XXV</td>
<td>Ala-Sar-Sar-Asp-Gly-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XXVI</td>
<td>Ala-Sar-Gly-Asp-Sar-Lys</td>
<td>NI</td>
</tr>
<tr>
<td>XXVII</td>
<td>Ala-Sar-Sar-Asp-Sar-Lys</td>
<td>50</td>
</tr>
</tbody>
</table>
Thus, our preliminary studies have resulted in the identification of peptides (XVI, XXI and XXIV) that could inhibit the classical pathway activity. Though the peptides exhibited complement inhibition at a much higher dose, it is of great significance that a small linear peptides comprising of only six amino acid residues could block the human complement. Such an activity in small molecule may have a potential as therapeutic agents since they can be synthesised in large quantities with a high order of purity. The lead generated by us can be used to either create libraries by combinatorial chemistry or to design peptidomimetics for the development of therapeutically useful compounds. Further, studies with combinatorial libraries of the above peptides are in progress.
PART - 2

INVESTIGATION OF INTER CELLULAR ADHESION MOLECULE-1 PEPTIDES FOR THE TREATMENT OF ASTHMA
A major characteristics of asthma is the extreme sensitivity of the bronchi to inhaled agents\textsuperscript{199,200}. The severity of this “airway hyperresponsiveness” correlates with the intensity of asthmatic symptoms\textsuperscript{200,202} and therapy required\textsuperscript{200,203}. Although the underlying pathogenic mechanisms are not known many studies suggest that eosinophil infiltration and desquamation of the bronchial epithelium are involved\textsuperscript{204,206}. Since eosinophil-derived mediators damage airway epithelial cells \textit{in vitro}, these two events may be linked\textsuperscript{207}.

Adhesion of leukocytes to microvascular endothelium is essential for their migration into inflamed tissues. Infected tissues in severely affected patients contain little or no neutrophil. A ligand for some of these receptors\textsuperscript{208,209} is inter cellular adhesion molecule-1 (ICAM-1). It was shown to be upregulated on endothelium and skin epithelium both \textit{in vitro} and \textit{in vivo} 4 to 24 hrs after an inflammatory stimulus\textsuperscript{209,211}. In addition, monoclonal antibodies (MAbs) to ICAM-1 attenuate neutrophil adhesion to endothelium and inhibit neutrophil trans endothelial migration \textit{in vitro}\textsuperscript{209-213} and \textit{in vivo}. Thus, antagonism of ICAM-1 may provide a therapeutic approach to reducing airway hyper reactivity, hyper-responsiveness and asthma symptoms.

Intercellular adhesion molecule-1 (ICAM-1) is a 505 residue glycoprotein localised on endothelial cells. ICAM-1 is a ligand/receptor for lymphocyte function-associated antigen-1 (LFA-1) located in the cell surface of leucocytes. The extra cellular region of ICAM-1 is predicted to contain five immunoglobulin like domains of the C2 type, a trans membrane region and a short cytoplasmic tail\textsuperscript{252,253}. On the basis of these data and other structure similarity ICAM-1 has been included in the immunoglobulin superf gene family.
6.1 Rational for the Synthesis of Inter Cellular Adhesion Molecule-1 (ICAM-1) Related Peptides

In order to investigate the role of various immunoglobulin segment of the entire extracellular domain of ICAM-1, which may mimic the inhibitory effects of the anti monoclonal antibody. We have synthesised five peptide segments located in the proposed immunoglobulin like domain-1 and 4. The sequence and domains of the five peptide synthesised have been shwon in fig. 1. All the peptide fragments were synthesized with the view to inhibit the ICAM-1 dependent process and may provide a therapeutic approach to reduce airway inflammation, hyper responsiveness and asthma symptoms.

6.2 Synthesis of Peptides (I-V)

All the peptides from I to V described in this chapter, were synthesised manually using solid phase method of peptide synthesis.\textsuperscript{220} Fmoc-chemistry\textsuperscript{221-223} was used to synthesise all peptides using methyl benzhydryl amine (MBHA) resin\textsuperscript{254}, as solid support to get peptide amides. The side chains of Glu, Asp, Thr and Ser was protected by acid labile t-butyl (Bu') group. The side chains of Lys and Arg was protected by acid labile t-butyloxy carbonyl (Boc)\textsuperscript{226} and 2,2,5,7,8-pentamethyl chroman-6-sulphonyl (Pmc)\textsuperscript{227} groups respectively. All the protecting groups used for side chain functionality have been chosen in a manner that they can be readily removed during the final cleavage of the peptide from the resin. The synthesis involved following steps.

6.2.1 Loading of C-terminal amino acid to the resin

For the loading the \textsuperscript{N} Fmoc-protected c-terminal amino acids was attached to methyl benzhydryl amine (MBHA) resin (prewashed with DCM, and 8% DIEA/DCM followed by DCM/DMF washes) by DIC/HOBt procedure (Scheme 1), for 3 hrs agitation under \textsubscript{N}$_2$ current. The completion of acylation was monitored using the Kaiser's test.
FIG. 1: The Sequences of Peptide Fragments I-V

Peptide I

- Sequence: Lys-Glu-Leu-Leu-Pro-Gly-Asn-Asn Arg-Lys-Val-NH$_2$
- Fragment No.: 40-51

Peptide II

- Sequence: Gln-Thr-Ser-Val-Ser-Pro-Ser-Lys-Val Ile-Leu-Pro-NH$_2$
- Fragment No.: 1-12

Peptide III

- Sequence: Gly-Asn-Asn-Arg-Lys-Val-Tyr-Glu Leu-Ser-Asn-Val-NH$_2$
- Fragment No.: 46-57

Peptide IV

- Sequence: Pro-Gly-Asn-Trp-Thr-Trp-Pro- Glu-Asn-Ser-Glu-Gln-Thr-Pro-NH$_2$
- Fragment No.: 379-392

Peptide V

- Sequence: Asn-Gln-Pro-Lys-Leu-Leu-Gly-Ile Glu-Thr-Pro-Leu-NH$_2$
- Fragment No.: 26-37
6.2.2 Deprotection

20% piperidine-DMF solution was used for the removal of Fmoc-group from the peptidyl resin during each coupling steps.

6.2.3 Couplings

In order to elongate the peptide chain, the subsequent Fmoc-amino acids was coupled with the free amino terminal of the peptidyl resin by diisopropyl carbodiimide (DIC/HOBt) method\textsuperscript{230,231}.

Deprotection and coupling steps were repeated for the attachement of the remaining Fmoc-amino acids in a successive steps until the desired peptide sequence was build up on the solid support. This was followed by coupling of N-terminal Boc-protected amino acids to peptidyl resin.

6.2.4 Final cleavage of the peptide

The cleavage of all the peptides (I-V) from the solid support was achieved by well established Two-step cleavage method using reagent K/TFMSA\textsuperscript{255}. In this procedure the ice cooled peptidyl resin (0.5 g) was treated with an ice cooled mixture of reagent K [phenol (0.75 g), thioanisole (1 ml), ethane dithiol (0.5 ml), water (0.5 ml) and TFA (10 ml)] in a 50 ml flask. The flask was stoppered and the contents were stirred at r.t. for 1.5 hr. The mix was then filtered and the resin was washed with TFA (2x2 ml). The resin was transferred to a 50 ml flask and treated with thioanisole (1 ml) and ethane dithiol (0.5 ml) followed by TFA (10 ml) and the mixture was stirred at room temperature. The mixture was then cooled in an ice bath and TFMSA (1 ml) was added dropwise with stirring. The flask was stoppered and stirred at room temperature for 1.5 hr. Ice cold diethyl ether (50 ml) was added and the mixture was stirred vigorously for 1 min and then filtered. The peptide was extracted into TFA (3x3 ml) and the solution was concentrated in vacuo and then treated with cold ether to precipitate the peptide. The crude peptide was purified on RPHPLC.
6.3 Synthetic Protocol Adopted for Solid Phase Peptide Synthesis

The general procedure adopted for the synthesis of compounds (I-V) is given in table 1. The volumes of the reagents are given for 250 mg of the resin loaded with the C-terminal amino acid.

All the steps described in table 1 were carried out under nitrogen current in an apparatus, especially designed for solid phase peptide synthesis. A gentle nitrogen current was used to agitate the resin in the presence of the reagents described in table 1, as well as to provide anhydrous atmosphere during the synthesis.

Table 1 : Standard Synthetic Protocol for Solid Phase Peptide Synthesis

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent</th>
<th>Volume (ml)</th>
<th>No. of Times</th>
<th>Time for each (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DMF</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>20% Piperidine-DMF</td>
<td>8</td>
<td>Once</td>
<td>15</td>
</tr>
<tr>
<td>3.</td>
<td>DMF</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>4.</td>
<td>iPrOH</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>DMF</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>Fmoc-aminoacid (3 molar excess)</td>
<td>8</td>
<td>Once</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>HOBt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3 molar excess)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>DMF</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>8.</td>
<td>MeOH</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>9.</td>
<td>DCM</td>
<td>8</td>
<td>Thrice</td>
<td>2</td>
</tr>
<tr>
<td>10.</td>
<td>Recoupled if positive Kaiser's test by repeating steps 5-9.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6.4 Monitoring of Coupling Reaction

All the coupling reactions were monitored by Kaiser's test\textsuperscript{232}. For this test a small aliquot of the peptide resin was taken in a sample tube and three drops of each solution i.e. $2 \times 10^{-4}$ mole KCN/Pyridine, 76% phenol/ethanol (w/w) and 0.28 mole ninhydrin/ethanol added to it. The sample was heated for 5-6 min at 100$^\circ$ C and the colour of the beads examined. Absence of the blue colouration of the resin beads indicated a negative Kaiser's test thereby confirming the completion of the coupling reaction.

6.5 Experimental

All the amino acids used were of L-configuration. All the reactions were carried out exclusively by using dry reactants and solvents. Homogeneity and purity of all the peptides were determined by TLC on silica gel-G plates using the following solvent systems.

A) n-BuOH-Pyridine-AcOH-H$_2$O (9:3:3:5)

B) n-BuOH-AcOH-H$_2$O (3:3:1)

C) n-BuOH-AcOH-Pyridine-H$_2$O (15:3:10:3)

The final purification and confirmation of the homogeneity of peptides was done with the help of reverse phase HPLC. The solvent system used for analytical HPLC was a binary gradient system of 20-30% acetonitrile water 0.1% TFA over 15 min at a flow rate of 1.5 ml/min. A C$_{18}$ $\mu$ bond pack column (7.8 x 300 mm) was used and detection was done at 220 nm.

Amino acid composition of final peptides was determined by hydrolysis of the sample \textit{in vacuo} in constant boiling 6N HCl for 22 hrs at 110$^\circ$C. Sample were then analysed using pico-Tag (Millipore) amino analyser. Toshniwal melting points
apparatus was used for determination of the uncorrected capillary melting points. Optical rotations were recorded on Autopol. Polarimeter III. The mass spectra of final purified peptides were characterised by their FABMS on Jeol JMS SX-102 FAB mass spectrometer.

6.6 Synthesis of Analogues I to V via Solid Phase Peptide Synthesis on MBHA resin using Fmoc-Strategy

The synthesis of analogues I-V using solid phase peptide synthesis on methyl benzhydryl amine (MBHA) resin, in order to get peptide amide was done using the synthetic protocol described earlier in this chapter. However, the physico-chemical characteristics of all the peptides was given below:

Lys-Glu-Leu-Leu-Pro-Gly-Asn-Asn-Arg-Lys-Val (I)

Fmoc-Val (0.15 g, 0.45 mmol) was loaded on MBHA resin (0.25 g, 0.15 mmol g⁻¹) in a manner described earlier in this chapter. The required Fmoc-amino acid viz. Fmoc-Lys (Boc), Fmoc-Arg(Pmc), Fmoc-Asn, Fmoc-Gly, Fmoc-Pro, Fmoc-Leu and Fmoc-Glu(Bu')-OH, successively added to it for condensation in a stepwise manner for assembling the complete peptide chain using the synthetic protocol described in table 1. The physico-chemical data of the final peptide is as follows:

Yield : 83%; m.p. 136°C; Rf : 0.63 (A), 0.53 (B), 0.43 (C) [α]D 22-15.00 (C0.20 MeOH); FABMS : 1266 [M+H]+, Amino acid analysis ; Lys 1.94 (2), Glu 0.94 (1), Leu 2.04 (2), Pro 0.97 (1), Gly 1.01 (1), Asp 1.91 (2), Arg 0.98 (1) Val 1.01 (1).

Gln-Thr-Ser-Val-Ser-Pro-Ser-Lys-Val-Ile-Leu-Pro (II)

Fmoc-Pro (0.15 g, 0.45 mmol) was loaded on MBHA (0.25 g, 0.15 mmol g⁻¹) resin in a similar manner described earlier in this chapter. The required Fmoc-amino acids/terminal Boc-amino acids viz. Fmoc-Leu, Fmoc-Ile, Fmoc-Val, Fmoc-Lys (Boc), Fmoc-Ser(Bu'), Fmoc-Thr(Bu') and Boc-Gln, successively added to it for
condensation in a stepwise manner for assembling the complete peptide chain using the synthetic protocol described in table 1. The physico-chemical data of the final peptide is as follows:

Yield : 85%; m.p. 193°C; Rf : 0.63 (A), 0.63 (B), 0.71 (C) [α]D 25 ^25-65.00 (C0.20 MeOH); FABMS : 1255 [M+H]^+, Amino acid analysis ; Glu 0.97 (1), Thr 1.02 (1), Ser 3.10 (3), Val 2.03 (2), Pro 1.95 (2), Ile 0.97 (1), Leu 1.02 (1) Lys 0.98 (1).


Fmoc-Val (0.15 g, 0.45 mmol) was loaded to MBHA resin (0.25 g, 0.15 mmol g⁻¹) in a similar manner described earlier in this chapter. The required Fmoc-amino acids/terminal Boc-amino acid viz Fmoc-Asn, Fmoc-Ser, Fmoc-Leu, Fmoc-Glu/Bu⁻ Fmoc-Tyr(Bu⁻), Fmoc-Val, Fmoc-Lys(Boc), Fmoc-Arg(Pmc)-OH, and Boc-Gly, Successively added to it for condensation in a stepwise manner for assembling the complete peptide chain using the synthetic protocol described in table 1. The physico chemical data of final peptide is as follows:

Yield : 88%; m.p. 214°C; Rf : 0.73 (A), 0.80 (B), 0.54 (C) [α]D 25-25.00 (C0.20 MeOH); FABMS : 1392 [M+H]^+, Amino acid analysis Gly 0.98 (1), Asp 3.09 (3), Arg 1.03 (1) Lys 1.04 (1), Val 2.04 (1), ; Tyr 0.94 (1), Glu (0.96 (1), Leu 0.97 (1), Ser 0.98 (1).

**Pro-Gly-Asn-Trp-Thr-Trp-Pro-Gly-Asn-Ser-Glu-Glu-Thr-Pro (IV)**

Fmoc-Pro (0.15 gm, 0.45 mmol) was loaded on MBHA resin (0.25 g, 015 mmol g⁻¹) in a similar manner described earlier in this chapter. The required Fmoc-amino acids/terminal Boc-amino acids viz, Fmoc-Thr (Bu⁻), Fmoc-Glu, Fmoc-Ser (Bu⁻), Fmoc-Asn, Fmoc-Glu (Bu⁻), Fmoc-Trp, Fmoc-Gly and Boc-Pro successively added to it for condensation in a stepwise manner for assembling the complete peptide chain using the synthetic protocol described in table 1. The physico-chemical data of the final peptide is given below:
Yield: 87%; m.p. 221°C; Rf: 0.74 (A), 0.70 (B), 0.57 (C) [α]D25-35.00 (C0.20 MeOH); FABMS: 1641 [M+H]+, Amino acid analysis Pro 3.08 (3), Thr 1.99 (2), Glu 2.94 (3), Asp 1.98 (2), Ser 1.01 (1), Trp 2.01 (2), Gly 0.96 (1).

Asp-Glu-Pro-Lys-Leu-Leu-Gly-Ile-Glu-Thr-Pro-Leu (V)

Fmoc-Leu (0.15 g, 0.45 mmol) was loaded on MBHA resin (0.25 g, 0.15 mmol g⁻¹) in similar manner described earlier in this chapter. The required Fmoc-amino acids/terminal Boc-amino acids viz. Fmoc-Pro, Fmoc-Thr (Bu¹), Fmoc-Glu(Bu¹), Fmoc-Trp, Fmoc-Asn, Fmoc-Gln, and Boc-Pro successively added to it for condensation in a stepwise manner for assembling the complete peptide chain using the synthetic protocol described in table 1. The physico-chemical data of the final peptide is as follows:

Yield: 85%; m.p. 234°C; Rf: 0.57 (A), 0.67 (B), 0.62 (C) [α]D25-50.00 (C0.20 MeOH); FABMS: 1323 [M+H]+, Amino acid analysis Leu 2.98 (3), Pro 1.97 (2), Thr 0.94 (1), Glu 2.01 (2), Ile 0.98 (1) Gly 0.96 (1), Lys 1.02 (1), Asp 0.98 (1).

6.7 Bioevaluation

The antiallergic/antiasthmatic activity of all the five fragments derived from inter cellular adhesion molecule-1 (ICAM-1) were evaluated in the Division of Pharmacology, Central Drug Research Institute, Lucknow. The procedure used for the bioevaluation of various compounds and the results obtained are briefly discussed below:

6.7.1 Materials and Methods

The preliminary Screening of synthetic peptides (I-V) was done for antiallergic/antiasthmatic bioassay by studying antiPCA (passive cutaneous anaphylaxis), mast cell stabilizing activity by intraperitoneally in a similar way as described in chapter 4 of the thesis.
6.8 Results and Discussion

The antiallergic activity of compounds (I-V) has been summarized in table 1. The results have been presented as % inhibition of passive cutaneous anaphylaxis (PCA) and % protection of mast cell degranulation (induced by compound 48/80) and compound with disodium cromoglycate a standard antiallergic drug used clinically.

Table 1: antiallergic activity of analogues I-V

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Compounds</th>
<th>% Protection* of PCA at 1 mg/kg i.p.</th>
<th>% Protection* of mast cell degranulation at 0.20 mg/kg i.p. x 5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Lys-Glu-Leu-Leu-Pro-Gly-Asn-Asn-Arg-Lys-Val-NH₂ (40-51)</td>
<td>61.4</td>
<td>51</td>
</tr>
<tr>
<td>II</td>
<td>Gln-Thr-Ser-Val-Ser-Pro-Ser-Lys-Val-Ile-Leu-Pro-NH₂ (1-12)</td>
<td>63.6</td>
<td>46</td>
</tr>
<tr>
<td>III</td>
<td>Gly-Asn-Asn-Arg-Lys-Val-Tyr-Glu-Leu-Ser-Asn-Val-NH₂ (46-57)</td>
<td>67.5</td>
<td>67</td>
</tr>
<tr>
<td>IV</td>
<td>Pro-Gly-Asn-Trp-Thr-Trp-Pro-Glu-Asn-Ser-Gln-Gln-Thr-Pro-NH₂ (379-392)</td>
<td>74.9</td>
<td>61</td>
</tr>
<tr>
<td>V</td>
<td>Asp-Gln-Pro-Lys-Leu-Leu-Gly-Ile-Glu-Thr-Pro-Leu-NH₂ (26-37)</td>
<td>72.5</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>DSCG*</td>
<td>77</td>
<td>84</td>
</tr>
</tbody>
</table>

*DSCG at 50 mg/kg, i.p. ; # Calculation of % protection was carried out using saline treated control as base line
In general all the peptides exhibited anti-PCA and mast cell stabilising activity. Peptide IV, corresponding to 379-392 of ICAM-1 was the most active compound with 74.9% protection of PCA at 1 mg/kg, which was equipotent to DSCG at 50 mg/kg. However, in the mast cell stabilising assay it was less active than DSCG. Other peptides corresponding to domain 1 and 4 though exhibited significant antiallergic activity were found to be less active than clinically used drug DSCG.

The antiasthmatic activity of compound III, IV and V was carried out in guinea pig by aerosol test. The results have been summarized in table 2.

### Table 2: Aerosol test in normal and sensitised guinea pigs by Egg albumin

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg) p.o.</th>
<th>% Protection Egg albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>1.0</td>
<td>62.8</td>
</tr>
<tr>
<td>IV</td>
<td>1.0</td>
<td>55.1</td>
</tr>
<tr>
<td>V</td>
<td>1.0</td>
<td>51.1</td>
</tr>
<tr>
<td>DSCG</td>
<td>50.0 (i.p.)</td>
<td>72.1</td>
</tr>
</tbody>
</table>

When sensitized guinea pigs were exposed to the antigen aerosol in the aerosol chamber, there was either prevention of anaphylaxis or prolongation of latent period of preconvulsion. analogue III exhibited highest inhibition to preconvulsions and was
slightly less active than DSCG. The studies suggest that peptides derived from ICAM-1, though exhibited significant antiasthamatic/antiallergic activity, they were less active than clinically used drug DSCG. The peptides reported herein can be used as lead compounds for generating therapeutically useful agents.
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**Patent**