Localization of a Domain in Apolipoprotein E with both Cytostatic and Cytotoxic Activity†

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ABSTRACT: Apoliprotein E (apoE) is a potent suppressor of interleukin 2- (IL2-) dependent T lymphocyte proliferation. In this study, we have used a range of monomeric and dimeric peptides encompassing amino acids 130−169 in human apoE to locate a region with both cytostatic and cytotoxic effects on IL2-dependent T lymphocytes. Monomeric peptides representing residues 130−149 or 130−155 inhibited the proliferation of the cells without causing loss of cell viability. However, cytostasis by a peptide representing the extended 130−169 domain or dimeric peptides of amino acids 141−155 or 141−149 was accompanied by potent cytotoxic activity. These results suggest that residues 141−149, which include the overlap between the functional peptides, are responsible for cytostasis and cytotoxicity. Complete ablation of both activities by the polyanionic agent heparin highlighted the important contribution of the positively charged amino acids in the 141−149 region to peptide bioactivity. Furthermore, the bioactive apoE peptides also had a relatively high helical content, suggesting that α-helical content is necessary for bioactivity. Cytotoxic apoE peptides were characterized by a high density of polar face positively charged residues together with a high nonpolar face hydrophobicity. This conclusion is supported by the reduced hydrophobicity and polar face positive charge density of the significantly less active $E2_{130-169}$ peptide. The cytotoxic apoE peptides are structurally similar to previously characterized class L lytic peptides. They do not, however, exert their cytotoxic activity by destabilizing membrane bilayers as is the case with the class L peptides, as evidenced by their minimal hemolytic activity. We conclude that amino acids 141−149 in apoE, when present in an amphipathic α-helix conformation, represent an immunoregulatory sequence which has either cytostatic or cytotoxic activity, depending upon nonpolar face hydrophobicity and polar face positive charge density.

Apolipoprotein E (apoE)[1] plays a major role in the regulation of cholesterol homeostasis by mediating clearance of cholesterol-rich lipoproteins from the blood (Mahley, 1988). In recent years, several "nontraditional" functions for apoE have emerged, including a potential role for apoE isoforms in the pathogenesis of the neurodegenerative disorder Alzheimer's disease (AD) (Corder et al., 1993; Rebeck et al., 1993; Strittmater et al., 1993b). In addition, apoE appears to be involved in the regulation of the immune system (Harmony et al., 1986; Mahley, 1988). The regulated biosynthesis of apoE by monocyte-derived macrophages, accessory cells important in the immune system, has provided support for this possibility (Basu et al., 1982; Mazzone et al., 1987). ApoE has profound inhibitory effects on the proliferation of T lymphocytes *in vitro*. Proliferation of mitogen-activated T lymphocytes, purified or in mixed populations of peripheral blood mononuclear cells (PBM), is inhibited by apoE (Avila et al., 1983; Pepe & Curtiss, 1986; Kelly et al., 1994). ApoE is also a potent inhibitor of the proliferation of interleukin 2- (IL2-) dependent T lymphocytes (Mistry et al., 1995), the differentiated products of mitogen-activated naive T cells (Cantrell & Smith, 1984; Taniguchi & Minami, 1993).

Information on the molecular basis of apoE's cytostatic effects has emerged from two studies in which synthetic peptides were used to define a domain in apoE between amino acids 141−155 as a sequence that contributes to its suppression of mitogen-activated T cells (Cardin et al., 1988; Dyer et al., 1991). It is unclear whether this domain is also responsible for apoE's suppression of IL2-dependent cell proliferation. Although proliferation of both types of T lymphocytes is suppressed by apoE, it is possible that inhibition is mediated by distinct structural domains. One piece of evidence which suggests that this may be the case is that mitogen-activated naive T cells and IL2-responsive cells have a different susceptibility to apoE isoforms (Harmony et al., 1995). The three major isoforms of apoE,

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[1] Abbreiations: AD, Alzheimer's disease; apo, apolipoprotein; GAG, glycosaminoglycans; IL2, interleukin 2; FBS, fetal bovine serum; LDL, low-density lipoproteins; SFM, serum-free medium; PBM, peripheral blood mononuclear cells; TdR, methyl[^{3}H]thymidine, TFE, 2,2,2-trifluoroethanol; PBS, pphosphate-buffered saline; LRP, LDL-receptor-related protein.

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apoE2, apoE3, and apoE4, are equally potent toward mitogen-activated T cells. However, the Cys-for-Arg substitution at residue 158 in the apoE2 isoform obviates, to a great extent, apoE suppression of IL2-dependent T cell proliferation.[2]

We focus here on the identification of a domain in apoE that contributes to its suppression of IL2-induced T cell proliferation. On the basis of the studies of Dyer et al. (1991), which showed that peptides have greater cytostatic activity in multimeric form, we utilized a range of both monomeric and dimeric synthetic peptides representing sequences in apoE in the region defined by residues 130–169. This region includes the previously reported immunoregulatory domain, one of two heparin-binding domains (Cardin et al., 1986; Weisgraber et al., 1986), and the domain responsible for binding of apoE to the low-density lipoprotein (LDL) receptor (Weisgraber et al., 1983; Lalazar et al., 1988). Unexpectedly, two distinct immunoregulatory activities emerged: the arrest of cell proliferation that was not correlated with a reduction in cell viability and that which was correlated with cell death.

EXPERIMENTAL PROCEDURES

Peptide Synthesis. All peptides were synthesized by the standard solid-phase method, using N^{α}-Boc-amino acids with benzyl-based side-chain protecting groups and *p*-methylbenzhydrylamine resin on an Applied Biosystems Model 430A peptide synthesizer, as described previously (Diccianni et al., 1991). The branched dimer of $E_{141-155}$ was synthesized by first coupling N^{α}-Boc-Lys (ϵ-Boc) to the resin and then simultaneously elongating the peptide chain at both α- and ϵ-NH_2 groups. Peptides were cleaved from the resin by using anhydrous HF to obtain peptide amides. Crude peptides (10–25 mg) were purified by reversed-phase HPLC on a Vydac C-18 semipreparative column (250 × 10 mm, 10 μm particle size, 300 Å pore size), using a gradient of 25–60% B (0.1% TFA–CH_3CN) in 0.1% TFA–H_2O over 60 min at a flow rate of 4.7 mL/min. Fractions containing pure peptide were concentrated in a Savant vacuum concentrator, combined, lyophilized in aliquots, and stored at −20 °C. The overall yields of peptides were 5–25%. The purity of all peptides was >96%, as determined by analytical reversed-phase HPLC on a Vydac C-18 column (250 × 4.6 mm, 5 μm particle size, 300 Å pore size). Peptide concentration was quantitated by amino acid analysis. The integrity of the peptides was verified by complete sequence analysis, using automated Edman degradation on an Applied Biosystems Model 470A sequenator.

Cell Culture. Complete medium consisted of either RPMI-1640 (Hazelton, Lenexa, KS) or Dulbecco's modified Eagle's medium (DMEM; Whittaker Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hazelton), 25 mM HEPES (pH 7.4), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.3 mg/mL L-glutamine (RPMI-FBS or DMEM-FBS). Cells were washed with RPMI-1640 containing penicillin, streptomycin, and L-glutamine (RPMI). Serum-free medium (SFM) consisted of RPMI containing 15 μg/mL insulin, 10 μg/mL transferrin, 0.5 μg/mL linoleic acid, 5×10^{-5} M β-mercaptoethanol, 5 μg/mL catalase, and 1 mg/mL bovine serum albumin. For mouse cell lines, medium was supplemented with 2 mM pyruvate.

Cells were maintained at 37 °C in a humidified 5% CO_2 incubator. The mouse IL2-dependent cytotoxic T cell line (CTLL-15G) was obtained from Dr. Kendall Smith (Dartmouth Medical School, Hanover, NH). CTLL-15G cells, maintained in DMEM–FBS plus 5% rat T cell growth factor (McCarthy et al., 1987), were cultured at 1×10^4 cells/mL and passaged within 48–72 h. Human IL2-dependent primary T cells were generated, as described previously (Mistry et al., 1995). Briefly, human PBM (2.5×10^6 cells/mL) were incubated at 37 °C for 72 h with 10 ng/mL OKT3 (Ortho Diagnostics, Raritan, NJ), washed three times with RPMI, resuspended in RPMI–FBS, and incubated without IL2 for 24 h prior to the addition of recombinant human IL2 (rIL2; Collaborative Research, Bedford, MA) and apoE peptides.

Cell Proliferation Assay. Cellular uptake and incorporation into DNA of methyl[^{3}H]thymidine (TdR, 6.7 Ci/mmol; ICN Biomedicals Inc., Irvine, CA), as a measure of cell proliferation, were determined by liquid scintillation counting of harvested cell cultures. CTLL-15G cells (1×10^4/0.24 mL) were cultured in SFM in flat-bottom microtiter plates for 24 h with 20 units/mL rIL2 in the absence or presence of peptides; 1 μCi of TdR was added to each well 4 h prior to harvest. Human IL2-dependent primary T cells (1×10^5/0.24 mL) were cultured in SFM for 48 h with 10 units/mL rIL2 in the absence or presence of peptides and pulsed with 1 μCi of TdR 8 h prior to harvest. All cells were harvested onto glass fiber filter paper with a multiple sample automated harvester (Cambridge Technology Inc., Watertown, MA). Cell proliferation data are represented as the mean and standard error of the mean (SEM) of % cpm TdR incorporated for triplicate cultures relative to control (no peptide) = (experimental cpm/control cpm) × 100. TdR incorporation of control cells (no peptide) was 26 600 ± 2700 cpm in eight experiments with CTLL-15G cells and 28 400 ± 7200 cpm in six experiments with human primary T cells. The IC_{50} values for each peptide, representing the concentration of peptide which results in 50% inhibition of proliferation relative to control obtained by interpolation of each dose–response curve, are reported as mean ± SEM for several independent experiments.

Chromium Release Assay. Cell viability was determined by a long-term chromium release assay (Grabstein, 1980). Cells (1×10^7) were labeled with 100–200 μCi of sodium [^{51}Cr]chromate ($Na_2{}^{51}CrO_4$; 10–35 mCi/mL; Amersham, Arlington Heights, IL) in 500 μL of DMEM–FBS for 4 h at 37 °C. After being labeled, the cells were washed once and incubated for 30 min in 50 mL of SFM to reduce spontaneous release of ^{51}Cr and then washed three times with RPMI. Cytotoxicity assays, established at the same time as cell proliferation assays, contained no TdR. At 24 h (CTLL-15G) or 48 h (human primary cells), 100 μL of conditioned medium was collected from each of triplicate wells, and ^{51}Cr was quantitated in a TM Analytic γ counter. The percentage of specific ^{51}Cr release (peptide-mediated lysis) was calculated as % specific release = [(experimental release − spontaneous release)/(total release − spontaneous release)] × 100. Total ^{51}Cr release was determined by lysis of the cells with 1% NP-40 (Sigma Chemical Co., St. Louis, MO).

[2] M. A. Clay, M. J. Mistry, M. Z. Kounnas, D. K. Strickland, and J. A. K. Harmony, submitted for publication.

Spontaneous release was the ^{51}Cr release from cells incubated with rIL2 but in the absence of peptide. In all experiments, the spontaneous release was ≤1.7% of the total ^{51}Cr release/h, which is comparable with values reported by others for long-term ^{51}Cr release assays (Russell, 1981; Garner et al., 1991). The LC_{50} for each peptide represents the concentration of peptide which resulted in 50% specific ^{51}Cr release by the cells and was obtained by interpolation of each dose–response curve. The LC_{50} values for each peptide are reported as mean ± SEM for several independent experiments.

Peptide Analysis. Circular dichroic (CD) spectra of the apoE peptides were recorded in the absence or presence of 2,2,2-trifluoroethanol (TFE; Sigma), a helix-enhancing solvent which stabilizes secondary structure (Barrow et al., 1992; Sonnichsen et al., 1992; Otvas et al., 1993). Spectra were obtained over the interval of 196–250 nm on a JASCO 710 spectropolarimeter, calibrated with d_{10}-camphorsulfonic acid and using 0.1 cm path-length quartz cells. The α-helical content of the apoE peptides was estimated by using the method of Morrisett et al. (1973), according to the equation % α-helix $([\theta]_{222} - 3000/-3600 - 3000) \times 100$. Mean residue ellipticity ($[\theta]_{222}$) was calculated as $[\theta]_{222} = (\theta_{222})(MRW)/10lc$, where θ_{222} is the measured ellipticity angle at 222 nm, MRW is the mean residue weight, l is the optical path length (cm), and c is the protein concentration (g/mL).

The helical wheel diagrams of the peptides were computer generated, using the previously described WHEEL program (Jones et al., 1992). This program orients the helix with its nonpolar face directed toward the top of the page. Peptide hydrophobicity values were calculated by averaging the hydrophobicity of the residues that map to the nonpolar face of the amphipathic helix, as described previously (Jones et al., 1992); values are expressed as mean hydrophobicity per residue of the nonpolar face.

Hemolysis Assay. Human or sheep erythrocytes were washed three times with gelatin veronal buffer (Sigma). Cells (5×10^6/mL) were incubated in gelatin veronal buffer (total volume 1 mL) for 4 h at 37 °C in the absence or presence of peptide. Cells were incubated in water to determine total hemoglobin release. After incubation, the cells were centrifuged (10 min at 1000*g*), and supernatants were analyzed for hemoglobin release by measuring absorbance at 413 nm. Results are reported as a percentage of total hemoglobin release and are the mean and standard deviation (SD) of triplicate determinations.

RESULTS

Comparison of the Biological Activity of ApoE Peptides. Monomeric and dimeric peptides corresponding to amino acid residues 130–169 in human apoE and subregions within this domain (Table 1) were synthesized. The effects of these peptides on the IL2-induced proliferation of mouse CTLL-15G cells or human primary T cells were evaluated. $E_{130-169}$ inhibited the proliferation of CTLL-15G cells (Figures 1A) with an IC_{50} of 5.8 ± 0.6 μM (n = 8). This IC_{50} is comparable to that determined for native apoE, ~2–3 μM (Mistry et al., 1995). However, suppression by the peptide, in contrast to that by apoE, was accompanied by a significant loss of cell viability, assessed by trypan blue exclusion. Quantitation of $E_{130-169}$-mediated cytotoxicity (Figure 1A), using a chromium release assay, gave an LC_{50} of 10.5 ± 1.0 μM (n = 8). The cytotoxic activity was independent of assay method, as similar results were obtained when lactate dehydrogenase release was measured. Human IL2-dependent primary T cells, like mouse CTLL-15G cells, were suscep-

Table 1: ApoE Synthetic Peptides

peptide	amino acid sequence[a]
$E_{130-169}$	TEELRVRLASHLRKLRKRLLRDADDLQK*R*LAVYQAGAREG[b]
$E_{130-149}$	TEELRVRLASHLRKLRKRLL
$E_{130-155}$	TEELRVRLASHLRKLRKRLLRDADDL
$E_{141-169}$	LRKLRKRLLRDADDLQKRLAVYQAGAREG
$E_{150-169}$	RDADDLQKRLAVYQAGAREG
$E_{141-155}$	LRKLRKRLLRDADDL
Branched $E_{(141-155)2}$	LRKLRKRLLRDADDL LRKLRKRLLRDADDL >KCONH$_2$
Tandem $E_{(141-155)2}$	LRKLRKRLLRDADDL-LRKLRKRLLRDADDL
$E_{211-243}$	GERLRARMEEMGRSTRDRLDEVKEQVAEVRAKL

[a] The single-letter amino acid code is used. [b] In peptide $E2_{130-169}$, C is substituted for *R* at residue 158, marked with an asterisk.

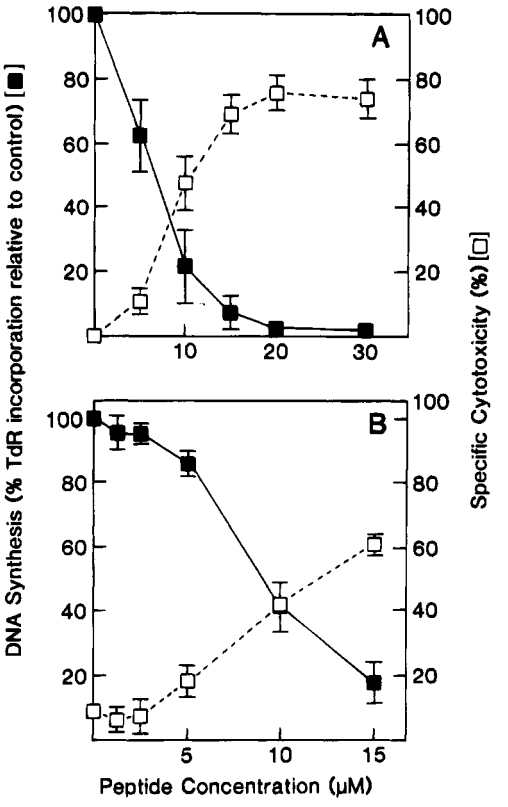

FIGURE 1: $E_{130-169}$ is cytostatic and cytotoxic to IL2-dependent T cells. The proliferation (■) of CTLL-15G cells (panel A) or human primary T cells (panel B) cultured in SFM with rIL2 (10 and 20 units/mL, respectively) and $E_{130-169}$ was measured, as described in Experimental Procedures. Values represent the mean percent TdR incorporation (±SEM) relative to control (no peptide) of three to four independent experiments performed in triplicate. Peptide cytotoxicity (□) was determined by a ^{51}Cr release assay, as described in Experimental Procedures. Values represent the mean percent specific cytotoxicity (±SEM) of three independent experiments performed in triplicate.

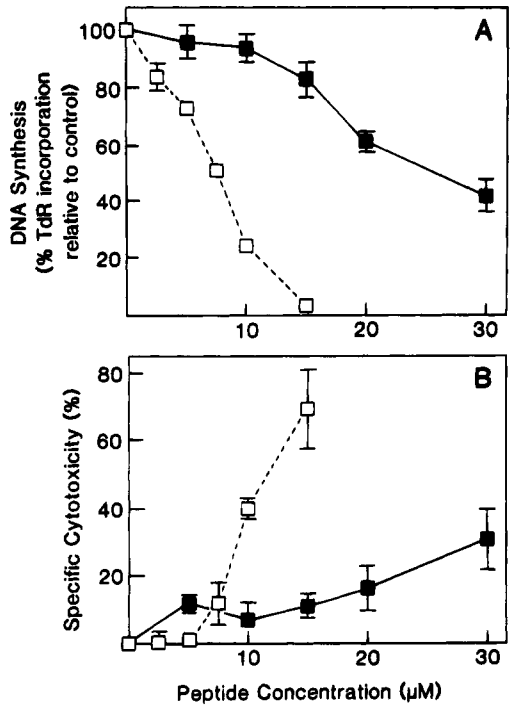

FIGURE 2: ApoE peptide activity is isoform specific. The proliferation of CTLL-15G cells cultured in SFM with 20 units/mL rIL2 and either $E2_{130-169}$ (■) or $E_{130-169}$ (□) was measured (panel A), as described in Experimental Procedures. Values represent the mean percent TdR incorporation (±SD) relative to control (no peptide) of a representative experiment performed in triplicate. Peptide cytotoxicity was determined by a ^{51}Cr release assay (panel B), as described in Experimental Procedures. Values represent the mean percent specific cytotoxicity (±SD) of a representative experiment performed in triplicate.

tible to both the suppressive (IC_{50} 8.7 ± 0.7 μM, $n = 6$) and cytotoxic (LC_{50} 12.4 ± 0.8 μM, $n = 6$) activities of $E_{130-169}$ (Figure 1B). The IC_{50} and LC_{50} values were not different when the amount of IL2 was increased from 10 to 2000 units/mL. Two conclusions can be drawn from these results: susceptibility to $E_{130-169}$ is not dependent upon cell adaptation to *in vitro* culture conditions, and it is not species specific.

To determine whether a Cys-for-Arg substitution at residue 158 would affect the activity of $E_{130-169}$, a peptide corresponding to amino acids 130−169 in apoE2 ($E2_{130-169}$) was synthesized (Table 1). This peptide also inhibited the IL2-dependent proliferation of CTLL-15G cells (Figure 2A). However, in two independent experiments, the IC_{50} (25.6 μM; $n = 2$) was 3−4-fold higher than that of $E_{130-169}$. Furthermore, incubation of the cells with $E2_{130-169}$ resulted in much lower levels of cytotoxicity (Figure 2B). $E2_{130-169}$ had less than 30% cytotoxicity at 30 μM, whereas $E_{130-169}$ resulted in approximately 70% cytotoxicity at 15 μM. These results indicate that, like native apoE, the bioactivity of the apoE peptides is isoform dependent.

To define further the bioactive subdomains within $E_{130-169}$, we asked whether the cytostatic and/or cytotoxic activities were localized in the amino or carboxyl half of the peptide. $E_{130-149}$ (Figure 3A) effectively suppressed IL2-induced proliferation of CTLL-15G cells with an IC_{50} of 15.1 ± 1.90 μM ($n = 4$). However, incubation of cells with this peptide resulted in only a minor loss of cell viability: 77% suppression of proliferation in the presence of 30 μM $E_{130-149}$ was accompanied by ~20% cytotoxicity. $E_{150-169}$ (Figure 3B) was neither suppressive nor cytotoxic, even at concentrations as high as 30 μM. A combination of $E_{130-149}$ (30 μM) and $E_{150-169}$ (30 μM) was not cytotoxic but resulted in an extent of inhibition of DNA replication comparable to 30 μM $E_{130-149}$ alone. In addition, $E_{141-155}$ (Figure 3C), the peptide encompassing the site connecting $E_{130-149}$ and $E_{150-169}$, was inactive.

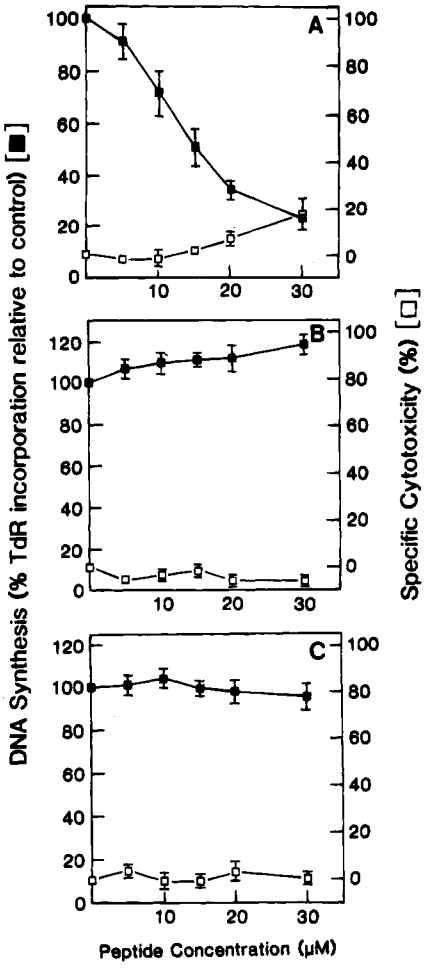

FIGURE 3: $E_{130-149}$ is cytostatic, but not cytotoxic, to IL-2-dependent T cells. The proliferation (■) of CTLL-15G cells cultured in SFM with 20 units/mL rIL2 and either $E_{130-149}$ (panel A), $E_{150-169}$ (panel B), or $E_{141-155}$ (panel C) was measured, as described in Experimental Procedures. Values represent the mean percent TdR incorporation (±SEM) relative to control (no peptide) of three independent experiments performed in triplicate. Peptide cytotoxicity (□) was determined by a ^{51}Cr release assay, as described in Experimental Procedures. Values represent the mean percent specific cytotoxicity (±SEM) of three independent experiments performed in triplicate.

Potent Cytotoxic Activity of Dimeric ApoE Peptides. We next asked whether peptide $E_{141-155}$, which displayed no activity as a monomer, is active when synthesized in dimeric form, as shown previously by Dyer et al. (1991) for mitogen-activated naive T cells. Dimeric apoE peptides representing two copies of amino acids 141−155 in either a branched (branched $E_{(141-155)_2}$) or tandem (tandem $E_{(141-155)_2}$) configuration were synthesized (Table 1). Both the branched (Figure 4A) and tandem (Figure 4B) peptide effectively suppressed the proliferation of CTLL-15G cells, with IC_{50}'s of 4.7 ± 1.0 μM ($n = 3$) and 1.9 ± 0.2 μM ($n = 3$), respectively. Suppression was accompanied by potent cytotoxic activity with LC_{50}'s of 15.5 ± 3.9 μM ($n = 3$) and 3.2 ± 0.4 μM ($n = 3$), respectively. The activities of these dimeric apoE peptides, like those of the apoE peptide

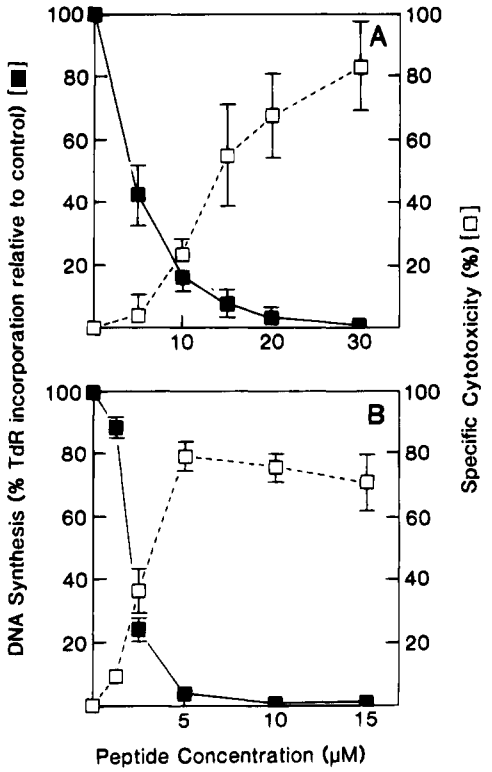

FIGURE 4: Dimers of amino acids 141−155 are cytostatic and cytotoxic to IL-2-dependent T cells. DNA replication (■) by CTLL-15G cells cultured in SFM with 20 units/mL rIL2 and either branched $E_{(141-155)_2}$ (panel A) or tandem $E_{(141-155)_2}$ (panel B) was measured, as described in Experimental Procedures. Values represent the mean percent TdR incorporation (±SEM) relative to control (no peptide) of two to three independent experiments performed in triplicate. Peptide cytotoxic activity (□) was determined by a ^{51}Cr release assay, as described in Experimental Procedures. Values represent the mean percent specific cytotoxicity (±SEM) of two to three independent experiments performed in triplicate.

monomers, were not influenced by the IL2 concentration. These data demonstrate that cytotoxicity is independent of the configuration of the peptide dimer. Consideration of the activities of the apoE peptides encompassed by residues 130−169 indicates that the 141−149 region, which represents the overlap between the functional peptides, is responsible for both cytostatic and cytotoxic effects. Therefore, we synthesized a dimeric peptide of 141−149 in the tandem configuration. This peptide was also cytostatic and cytotoxic, with IC_{50} and LC_{50} values about 20% higher than those of the tandem dimer of 141−155.

Dyer et al. (1991) previously reported that a monomeric peptide of the 141−155 sequence in apoE has no biological activity against mitogen-activated T cells, whereas the corresponding tandem peptide is cytostatic but not cytotoxic. The disparity between the cytotoxic effects of this peptide in the two cell systems is unclear. The major difference between the experimental designs in the two studies is the inclusion by Dyer et al. (1991) of FBS in the medium; in our experiments, cells were cultured in the absence of FBS. Amphipathic peptides can interact with lipoproteins present in FBS, and this may explain the difference. However, we have found that tandem $E_{(141-155)_2}$ is also cytotoxic in the presence of FBS (data not shown). Dyer et al. (1991) examined peptide cytotoxicity by using the lactate dehydrogenase (LDH) release assay. FBS contains fairly high levels of endogenous LDH. The LDH contributed by dying cells may therefore have been a small undetected proportion of the total. Since our peptides were purified and well characterized, we are confident that apoE peptide bioactivity was not due to an impurity. In addition, exhaustive dialysis of the peptides prior to use did not eliminate their cytotoxic activity. ApoE peptide cytotoxicity was also not an artifact of the synthesis procedure, since other peptides, representing different sequences but of a similar length and synthesized by the same procedure, had no effect on IL2-dependent cells.

Comparison of the IC_{50}'s of $E_{130-149}$ and $E_{(141-155)_2}$ shows that dimers of the sequence $E_{141-155}$ are 3−7 times more suppressive than $E_{130-149}$. To determine whether this difference is due primarily to the existence of a potent suppressive signal within residues 150−155, peptide $E_{130-155}$ was synthesized and tested for biological activity. $E_{130-155}$ had cytostatic and cytotoxic activities comparable to those of $E_{130-149}$: its IC_{50} was ~22 μm and its LC_{50} >30 μm. However, $E_{141-169}$ was neither suppressive nor cytotoxic at concentrations as high as 40 μM.

Structural Basis of ApoE Peptide Cytotoxicity. The cytotoxic activity of the apoE peptides was intriguing, as cytotoxicity is not a known characteristic of native apoE. Other peptide toxins, such as those present in wasp and bee venom and synthetic peptides of the human immunodeficiency virus type-1 envelope glycoprotein, have a high α-helical content (Argiolas & Pisano, 1984; DeGrado, 1988; Segrest et al., 1990; Srinivas et al., 1993). Therefore, we asked whether apoE peptide cytotoxicity were related to helical content. The $[\theta]_{222}$ of each apoE peptide was assessed by circular dichroism (CD) analysis in the absence and presence of TFE, and the α-helical content was estimated by the method of Morrisett et al. (1973). TFE is a membrane-mimicking solvent which is widely used to stabilize α-helices in regions with some helical propensity in peptides which are otherwise unstructured in aqueous solutions; TFE does not induce helix formation independently of sequence (Barrow et al., 1992; Sonnichsen et al., 1992; Otvas et al., 1993). Although the α-helical content of a peptide in TFE does not necessarily correlate with that during an incubation with cells, it does indicate the propensity of a peptide to form an α-helix. The cytostatic peptide $E_{130-149}$ could not be distinguished from the cytotoxic apoE peptides on the basis of its α-helical content. The helical content of all but one of the bioactive peptides was 31−46% in PBS. These values increased to 60−81% in the presence of TFE (Table 2). By contrast, the helical contents of $E_{150-169}$ and $E_{141-155}$ were 37% and 44%, respectively, in the presence of TFE, indicating that these inactive apoE peptides had a significantly lower overall propensity to form an α-helix. The exception was branched $E_{(141-155)_2}$, which is potently suppressive and cytotoxic, yet had a propensity to form an α-helix similar to that of the inactive peptides.

Helical wheel diagrams of bioactive apoE peptides (Figure 5) were constructed to determine whether apoE peptide cytotoxicity were related to unique features of the peptides' predicted amphipathic helical domains. The cytotoxic peptides $E_{130-169}$ and tandem $E_{(141-155)_2}$ (Figure 5A,C) have higher nonpolar face hydrophobicities (0.70 and 0.82 vs 0.64, respectively) and a higher number of polar face positive residues compared to the cytostatic peptide $E_{130-149}$ (Figure 5B) (9 and 11 vs 7, respectively; Table 2). It is interesting that the Cys-for-Arg substitution at residue 158 in $E2_{130-169}$

Table 2: Properties of Active and Inactive ApoE Peptides[a]

peptide	residues	α-helix in TFE, %	nonpolar face hydrophobicity	polar face positive residues	IC_{50}, μM	LC_{50}, μM
bioactive peptides						
$E_{130-169}$	40	67	0.70	9	5.8 ± 0.6	10.5 ± 1.0
$E2_{130-169}$	40	60	0.62	8	25.6	>30
$E_{130-149}$	20	81	0.64	7	15.1 ± 1.9	>30
$E_{130-155}$	26	74	0.71	6	22.0	>30
branched $E_{(141-155)_2}$	31	38	0.85	8	4.7 ± 1.0	15.5 ± 3.9
tandem $E_{(141-155)_2}$	30	79	0.82	11	1.9 ± 0.2	3.2 ± 0.4
inactive peptides						
$E_{141-169}$	29	59	0.68	8	>40	NC
$E_{150-169}$	20	37	0.42	4	NS	NC
$E_{141-155}$	15	44	0.85	5	NS	NC
$E_{211-243}$	33	32	0.74	9	NS	NC

[a] NS = nonsuppressive; NC = noncytotoxic (tested at concentrations to 40 μM).

(*, Figure 5B) only slightly reduces the nonpolar face hydrophobicity from 0.70 to 0.62 and the number of polar face positive residues from 9 to 8 but is associated with a profound reduction in both cytostatic and cytotoxic potency. In terms of their nonpolar face hydrophobicities and levels of positive charge, the cytotoxic apoE peptides are similar to the previously described class L lytic peptides (Segrest et al., 1990). However, according to the recent findings of Tytler et al. (1993), the presence of interfacial Arg residues and the high Arg:Lys ratio of $E_{130-169}$ and tandem $E_{(141-155)_2}$ prevent them from forming the "inverted" wedge conformation, which is characteristic of class L peptides. The properties of the apoE peptides are summarized in Table 2.

Heparin Ablates ApoE Peptide Activity. Analysis of the activities of the apoE peptides encompased by residues 130−169 in apoE suggests that the 141−149 region of apoE is important for activity. An obvious feature of this sequence is the presence of four positive charges. We reasoned that if the positive charge of this domain were responsible for peptide-mediated cytostasis and cytotoxicity, polyanionic agents such as heparin should ablate both activities. The effects of heparin on $E_{130-149}$-mediated cytostasis (Figure 6A) and cytotoxicity due to tandem $E_{(141-155)_2}$ (Figure 6B) were evaluated. The concentrations of $E_{130-149}$ and tandem $E_{(141-155)_2}$ used in the incubations were equivalent to the IC_{50}'s and LC_{50}'s, respectively. Heparin at 100 μg/mL maintained cell proliferation by cells incubated with $E_{130-149}$ at near control levels. The cytotoxic effect of tandem $E_{(141-155)_2}$ was completely ablated in the presence of 10 μg/mL heparin. As expected, DNA replication by cells incubated with the tandem peptide was also maintained at near control levels by heparin. In addition, preincubation of the cells with heparin followed by its removal during incubation with peptide had no effect on peptide activity, suggesting that heparin did not alter the cells' susceptibility to the peptides. Chondroitin sulfate similarly relieved apoE peptide suppression and cytotoxicity. Heparin or chondroitin sulfate alone had minimal effect on IL2-dependent cell proliferation.

We also determined the effects of a positively charged peptide representing amino acids 211−243, the other heparin-binding domain in apoE (Cardin et al., 1986), on IL2-dependent T cell proliferation and viability. $E_{211-243}$ (Figure 5D) has a polar face positive charge density of nine residues, which is midway between that of the cytostatic peptide $E_{130-149}$ and the cytotoxic peptides $E_{130-169}$ and tandem $E_{(141-155)_2}$, and a nonpolar face hydrophobicity of 0.74. However, concentrations of $E_{211-243}$ up to 40 μM had no effect on cell proliferation or viability. The results, taken together, indicate that the positively charged 141−149 sequence, the domain which represents the overlap between the functional $E_{130-169}$ and $E_{(141-155)_2}$ peptides, is responsible for cytostasis and cytotoxicity.

Cytotoxic ApoE Peptides Are Not Hemolytic. The predicted structural properties of the amphipathic helical segments of the cytotoxic apoE peptides, $E_{130-169}$ and tandem $E_{(141-155)_2}$, suggest that their cytotoxic effects on T lymphocytes were not due to destabilization of the cell membrane resulting in cell lysis. In order to test whether the apoE peptides had the ability to disrupt membranes, the hemolytic activities of the apoE peptides were compared with that of mellitin, a 26-residue peptide toxin isolated from honeybee venom which is potently hemolytic (Tosteson & Tosteson, 1981; DeGrado, 1988). Concentrations of tandem $E_{(141-155)_2}$ as high as 30 μM did not lyse erythrocytes during a 4 h incubation, whereas 30 μM mellitin caused 100% lysis (Figure 7). A similar result was obtained using sheep erythrocytes incubated for either 4 or 24 h. $E_{130-169}$ did cause approximately 50% hemolysis, but at a concentration 3-fold higher than the LC_{50} determined for T cell cytotoxicity. The apoE peptides cytotoxic to T cells had no effect on the viability of human liver HepG2 cells, human uterine epithelial Ishikawa cells, or human fibroblasts (data not shown).

DISCUSSION

Amino acids 130−169 in human apoE encompass an immunoregulatory domain with both cytostatic and cytotoxic activities against IL2-dependent T cells. This finding is consistent with results of previous studies (Cardin et al., 1988; Dyer et al., 1991) that implicated residues 141−155 in apoE's antiproliferative effect on naive mitogen-activated T cells. The similar potencies of $E_{130-149}$ and $E_{130-155}$ indicate that the cytostatic domain is located within residues 130−149. However, a longer peptide representing residues 130−169 and dimeric peptides of amino acids 141−155 also have potent cytotoxic activity. These results indicate that the positively charged, leucine-rich sequence, corresponding to amino acids 141−149-RKLRKRLL- in the mature protein which represents the overlap between the functional peptides identified, is responsible for both the cytostatic and cytotoxic effect.

The density of positively charged amino acid residues in the 141−149 domain clearly makes a significant contribution

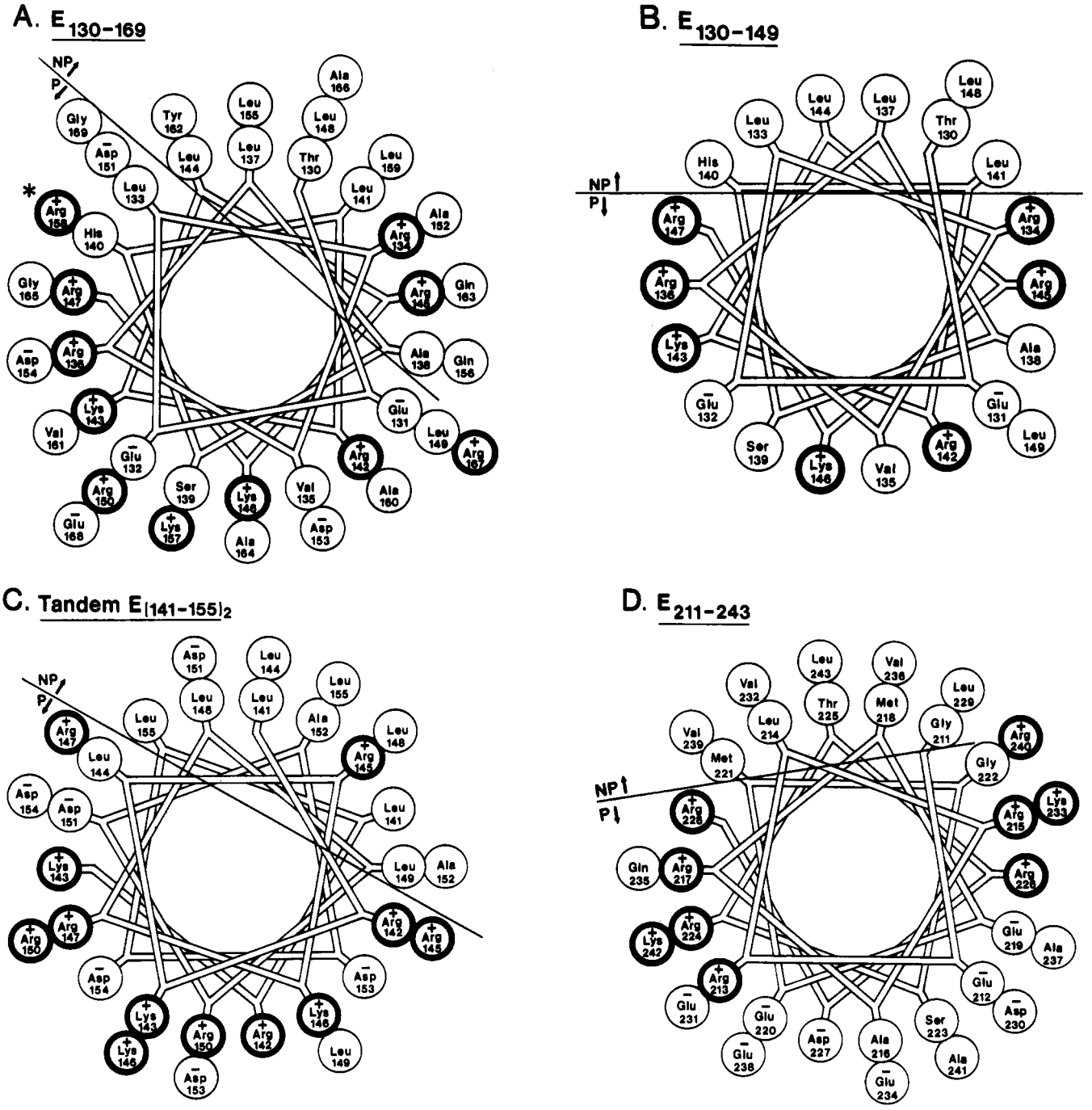

FIGURE 5: Helical wheel diagrams of apoE peptides. Helical wheel diagrams of the $E_{130-169}$ (panel A), $E_{130-149}$ (panel B), tandem $E_{(141-155)_2}$ (panel C), and $E_{211-243}$ (panel D) were computer generated by using the WHEEL program. Each peptide was modeled with the Lys and Arg residues in snorkel orientation. Positively and negatively charged amino acid residues are highlighted, and the position of the nonpolar–polar interface is marked.

to apoE peptide bioactivity. Consistent with this conclusion is the ablation of both peptide-mediated cytostasis and cytotoxicity by the polyanionic glycosaminoglycans (GAG), heparin and chondroitin sulfate. GAG-binding capacity does not, in itself, account for bioactivity since peptide $E_{211-243}$, which can bind heparin (Cardin et al., 1988) but which lacks the 141−149 sequence, is inactive. Calculation of an electrostatic potential map for the amino-terminal domain of human apoE indicates a large region of positive potential projecting out from residues 136−150 (Wilson et al., 1991). It is therefore possible that, in the bioactive monomeric apoE peptides, the additional positive charge of amino acids 136−140 is required for the 141−149 sequence to express immunoregulatory activity. The lack of activity of $E_{141-169}$ supports this proposal. Amino acids 136−140 are not, however, necessary for bioactivity as evidenced for the potent activity of the 141−155 and 141−149 dimeric peptides which do not contain this sequence. The importance of positive charge density for apoE peptide bioactivity is highlighted by the reduced activity of $E2_{130-169}$ compared to $E_{130-169}$. Studies of the three-dimensional structure of human apoE have shown that substitution of Cys for Arg at position 158 disrupts the region of positive charge in the vicinity of the 141−149 region, despite the fact that residue 158 is well removed from the 141−149 sequence (Wilson et al., 1991).

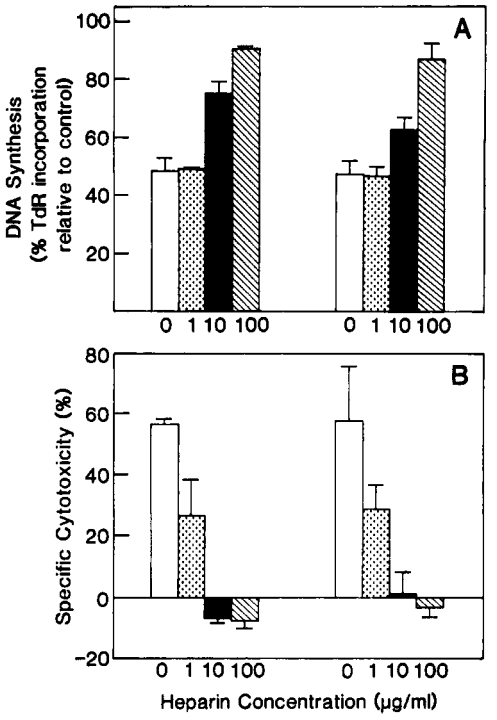

FIGURE 6: Heparin ablates the cytostatic and cytotoxic activities of apoE peptides. The proliferation (panel A) of CTLL-15G cells cultured in SFM with 20 units/mL rIL2, $E_{130-149}$ (15 μM), and increasing concentrations of heparin (grade I from porcine intestinal mucosa, Sigma) was measured, as described in Experimental Procedures. Bars represent the mean percent TdR incorporation (±SD) relative to the appropriate control, containing heparin but no peptide, in a representative experiment performed in triplicate. The cytotoxicity of tandem $E_{(141-155)_2}$ (3.25 μM) toward CTLL-15G cells cultured with IL2 and heparin (panel B) was determined by a ^{51}Cr release assay, as described in Experimental Procedures. Bars represent the mean percent specific cytotoxicity (±SD) in a representative experiment performed in triplicate.

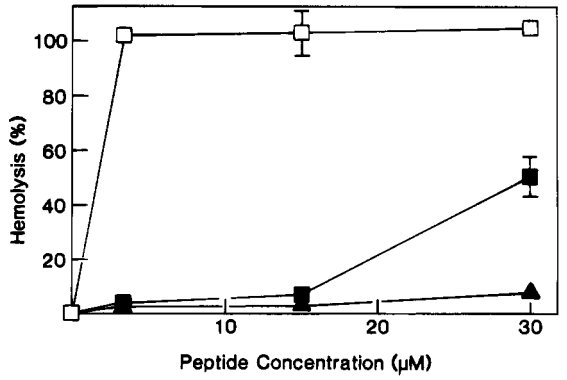

FIGURE 7: ApoE peptides have low hemolytic activity. Human erythrocytes, suspended in gelatin veronal buffer, were exposed to increasing concentrations of $E_{130-169}$ (■), tandem $E_{(141-155)_2}$ (▲), or the bee venom toxin mellitin (Sigma; □). After a 4 h incubation at 37 °C, the amount of hemoglobin release was determined spectrophotometrically, as described in Experimental Procedures. Values represent the mean percent of maximum lysis (±SD) in a representative experiment, performed in triplicate. Hemoglobin release by cells incubated in water for 4 h was considered to be 100% lysis.

The contribution of positively charged residues in the 141−149 region to apoE peptide bioactivity is likely to relate to the interaction between the peptides and the cells. It is not known whether peptide-mediated cytostasis and cytotoxicity are receptor-mediated effects. However, the logical extension of the finding that the cytotoxic apoE peptides have low hemolytic activity is the presence of a specific site of interaction for the peptides at the cell surface. An obvious candidate for an "immunoregulatory" binding site is the LDL receptor, as the LDL receptor binding domain of apoE is located in residues 140−160 (Weisgraber et al., 1983; Mahley, 1988; Lalazar et al., 1988). However, the absence or presence of the high-affinity LDL receptor does not influence apoE peptide suppression or cytotoxicity, suggesting that this receptor is not involved.[2] The LDL receptor-related protein (LRP) is also a potential binding site for the apoE peptides. The domain of apoE responsible for binding to the LRP, although not yet determined, has been predicted to involve residues 140−160. Also, multiple copies of apoE appear to be required for binding to one LRP (Beisiegel et al., 1989; Kowal et al., 1989). The reduced suppressive and cytotoxic activity of $E2_{130-169}$ provides support for the proposal that the LRP is the binding site for the apoE peptides, since previous studies have indicated that the Cys-for-Arg substitution at residue 158 in the E2 isoform reduces its binding affinity for the LRP (Kowal et al., 1990). We propose that the positively charged bioactive apoE peptides mediate their effects by binding to at least one of the negatively charged ligand-binding domains of the LRP.

An additional structure feature of the apoE peptides which appears to influence their interaction with the cells is their amphipathic α-helical conformation. Helical wheel analysis indicated that all of the bioactive peptides have the potential to assume an amphipathic α-helical structure, with a relatively high nonpolar face hydrophobicity and positive charge polar face density. Both positive charge and hydrophobicity enhance a peptide's affinity for negatively charged cell surfaces (Segrest et al., 1990; Taffs & Sitkovsky, 1992). Correlative evidence that α-helical content is necessary but not sufficient for bioactivity was obtained from CD analysis of the apoE peptides by which all of the active peptides exhibited a relatively high propensity to form an α-helix. This result is consistent with previous findings (Wilson et al., 1991) that residues 130−164 in human apoE form one of the four amphipathic α-helices in the amino-terminal domain of the protein. It is probable that the short length of the 141−155 monomer precluded it from forming a stable α-helix, which may account for its lack of bioactivity. One exception to the correlation of peptide bioactivity with helicity is the diminished potential of the branched dimer of the 141−155 sequence, which has potent activity, to form a stable α-helix relative to its tandem dimer counterpart and the other bioactive apoE peptides. The reduced helical content of the branched peptide may relate to the structural constraint of the Lys residue linking the two copies of the 141−155 sequence.

The nonpolar face hydrophobicity and polar face positive charge of the cytotoxic apoE peptides are notably high when compared to the cytostatic 130−149 and 130−155 peptides. We suggest that cytotoxicity is a function of hydrophobicity and positive charge density and may, as a consequence, result from efficient peptide−cell interactions. The cationic class L peptide toxins described by Segrest et al. (1990), which have some properties in common with the apoE peptides, are highly surface active. However, we have ruled out the possibility that apoE peptides exert their cytotoxic activity by the same mechanism as the class L peptides. Class L peptides interact with membrane bilayers to produce dramatic

structural changes which result in the cell lysis (Argiolas & Pisano, 1984; Christensen et al., 1988; Srinivas et al., 1992). This does not appear to be the case with the cytotoxic apoE peptides, as evidenced by their low hemolytic activity. The apoE peptides have interfacial Arg residues and a high Arg:Lys ratio, which prevent formation of the "inverted wedge" to promote the destabilization of membrane bilayers (Tytler et al., 1993). High concentrations of $E_{130-169}$ may cause some erythrocyte lysis due to the known ability of this peptide to bind lipid (Sparrow et al., 1985), allowing it to associate nonspecifically with cell membranes.

The identification of residues 141−149 in apoE as an immunoregulatory domain with both cytostatic and cytotoxic activities raises the question of whether peptide-mediated cytostasis and cytotoxicity are related cellular processes. Native apoE exerts its antiproliferative effects on IL2-dependent T cells by inhibiting cell cycle progression through the G1a/G1b boundary (Mistry et al., 1995). It is reasonable to suggest that the 130−149 and 130−155 peptides have a similar effect, since these peptides mimic the cytostatic activity of the native protein. We propose that the cell death caused by the cytotoxic apoE peptides is preceded by growth arrest. Close examination of the dose−response curves for each of the cytotoxic peptides shows that, at low concentrations of peptide, there is inhibition of proliferation which is not accompanied by significant cytotoxicity. However, at higher peptide concentrations, generally 2−3 times the IC_{50} for each peptide, suppression of proliferation can, in large part, be attributed to cell death.

The physiological relevance of the cytotoxic activity of the apoE peptides is uncertain. Native apoE is not cytotoxic at concentrations which are potently cytostatic toward both mitogen-activated and IL2-dependent T cells (Kelly et al., 1994; Harmony et al., 1995). However, the findings of two recent studies permit speculation that apoE expresses cytotoxic activity *in vivo*. A significant proportion of the apoE that is newly synthesized by either HepG2 cells (Ye et al., 1993) or a mouse macrophage cell line transfected with a constitutively expressed human apoE cDNA (Mazzone et al., 1992) is degraded intracellularly, allowing *in situ* generation of apoE peptides from the nascent protein. Parallels can be drawn between the apoE peptides and the bioactive peptide amyloid β (Aβ) which is released by proteolytic cleavage of the integral membrane protein β-amyloid precursor protein (β-APP) (Shoji et al., 1992; Haass et al., 1992). The Aβ fragment is the major constituent of amyloid plaques in the brains of subjects with AD and has been reported to have significant neurotoxic activity (Yankner et al., 1990; Kowal et al., 1991). Interestingly, apoE exhibits high-avidity binding for Aβ (Wisniewski et al., 1993; Strittmatter et al., 1993b) and colocalizes with Aβ in plaques and tangles in AD brain tissue (Wisniewski & Frangione, 1992). Furthermore, the gene encoding one of the isoforms of apoE (ϵ4) is associated with late-onset familial and sporadic AD (Corder et al., 1993; Rebeck et al., 1993; Strittmatter et al., 1993a). These recent findings raise the possibility that native apoE has a toxic activity *in vivo* which contributes to the extensive neurodegeneration found in this disease.

In summary, the fact that apoE peptide-mediated cytostasis mimics the activity of native apoE provides a versatile tool for examining mechanisms which regulate growth factor-dependent T cell proliferation. The unique cytotoxic activity of the apoE peptides that we have described here not only has far-reaching implications in terms of regulation of the T cell-mediated immune response but may also explain other observations such as the recently proposed role of apoE in the pathogenesis of neurodegenerative diseases (Roses, 1994).

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REFERENCES

Argiolas, A., & Pisano, J. J. (1984) *J. Biol. Chem. 259*, 10106−10111.

Avila, E. M., Holdsworth, G. Sasaki, N., Jackson, R. L., & Harmony, J. A. K. (1982) *J. Biol. Chem. 257*, 5900−5909.

Barrow, C. J., Yasuda, A., Kenny, P. T. M., & Zagorski, M. G. (1992) *J. Mol. Biol. 225*, 1075−1093.

Basu, S. K., Ho, Y. K., Brown, M. S., Bilheimer, D. W., Anderson, R. G. W., & Goldstein, J. L. (1982) *J. Biol. Chem. 257*, 9788−9795.

Beisiegel, U., Weber, W., Ihrke, G., Herz, J., & Greten, H. (1989) *Nature 341*, 162−164.

Cantrell, D. A., & Smith, K. A. (1984) *Science 224*, 1312−1316.

Cardin, A. D., Hirose, N., Blankenship, D. T., Jackson, R. L., & Harmony, J. A. K. (1986) *Biochem. Biophys. Res. Commun. 134*, 783−789.

Cardin, A. D., Bowlin, T. L., & Krstenansky, J. L. (1988) *Biochem. Biophys. Res. Commun. 154*, 741−745.

Christensen, B., Fink, J., Merrifield, R. B., & Mauzerall, D. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 5072−5076.

Corder, E. H., Saunders, A. M., Stritmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., & Pericak-Vance, M. A. (1993) *Science 261*, 921−923.

DeGrado, W. F. (1988) *Adv. Protein Chem. 39*, 51−124.

Diccianni, M. B., Lilly-Stauderman, M., McLean, L. R., Balasubramaniam, A., & Harmony, J. A. K. (1991) *Biochemistry 30*, 9090−9097.

Dyer, C. A., Smith, R. S., & Curtiss, L. K., (1991) *J. Biol. Chem. 266*, 15009−15015.

Garner, C. M., Mills, C. O., Elias, E., & Neuberger, J. M. (1991) *Biochim. Biophys. Acta 1091*, 41−45.

Grabstein, K. (1980) in *Selected Methods in Cellular Immunology* (Mishell, B. B., & Shiigi, S. M., Eds.) pp 124−137, W. H. Freeman & Co., San Francisco.

Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., Teplow, D. B., & Selkoe, D. J. (1992) *Nature 359*, 322−327.

Harmony, J. A. K., Akeson, A. L., McCarthy, B. M., Morris, R. E., Scupham, D. W., & Grupp, S. A. (1986) in *Biochemistry and Biology of Plasma Lipoproteins* (Scanu, A. M., & Spector, A. A., Eds.) pp 403−451, Marcel Dekker, Inc., New York.

Harmony, J. A. K., Kelly, M. E., Clay, M. A., & Mistry, M. J. (1995) *Exp. Med. Intl. Congr. Ser.* (in press).

Jones, M. K., Anantharamaiah, G. M., & Segrest, J. P. (1992) *J. Lipid Res. 33*, 287−296.

Kelly, M. E., Clay, M. A., Mistry, M. J., Hsieh-Liu, H.-M., & Harmony, J. A. K. (1994) *Cell. Immunol. 159*, 124−139.

Kowal, R. C., Herz, J., Goldstein, J. L., Esser, V., & Brown, M. S. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 5810−5814.

Kowal, R. C., Herz, J., Weisgraber, K. H., Mahley, R. W., Brown, M. S., & Goldstein, J. L. (1990) *J. Biol. Chem. 265*, 10771−10779.

Kowal, N. W., Beal, M. F., Busciglio, J., Duffy, L. K., & Yankner, B. A. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 7247−7251.

Lalazar, A., Weisgraber, K. H., Rall, S. C., Jr., Giladi, H., Innerarity, T. L., Levanon, A. Z., Boyles, J. K., Amit, B., Gorecki, M., Mahley, R. W., & Vogel, T. (1988) *J. Biol. Chem. 263*, 3542−3545.
Mahley, R. W. (1988) *Science 240*, 622−630.
Mazzone, T., Pustelnikas, L., & Reardon, C. A. (1992) *J. Biol. Chem. 267*, 1081−1087.
Mistry, M. J., Clay, M. A., Kelly, M. E., Steiner, M. A., & Harmony, J. A. K. (1995) *Cell. Immunol. 160*, 14−23.
Morrisett, J. D., David, J. S. K., Pownall, H. J., & Gotto, A. M. (1973) *Biochemistry 12*, 1290−1299.
Otvos, L., Svendri, G. I., Lee, V. M.-Y., & Mantsch, H. H. (1993) *Eur. J. Biochem. 211*, 249−257.
Pepe, M. G., & Curtiss, L. K. (1986) *J. Immunol. 136*, 3716−3723.
Rebeck, G. W., Reiter, J. S., Strickland, D. K., & Hyman, B. T. (1993) *Neuron 11*, 575−580.
Roses, A. D. (1994) *J. Neuropathol. Exp. Neurol. 53*, 429−437.
Russell, S. W. (1981) in *Methods for Studying Mononuclear Phagocytes* (Adams, D. O., Edelson, P. J., & Koren, H., Eds.) pp 793−799, Academic Press Inc., New York.
Segrest, J. P., Loof, H. D., Dohlman, J. G., Brouilette, C. G., & Anantharamaiah, G. M. (1990) *Proteins 8*, 103−117.
Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. L., Cai, X.-D., McKay, D. M., Tintner, R., Frangione, B., & Younkin, S. G. (1992) *Science 258*, 126−129.
Sonnichsen, F. D., Van Eyk, J. E., Hodges, R. S., & Sykes, B. D. (1992) *Biochemistry 31*, 8790−8798.
Sparrow, J. T., Sparrow, D. A., Culwell, A. R., & Gotto, A. M. (1985) *Biochemistry 24*, 6984−6988.
Srinivas, S. K., Srinivas, R. V., Anantharamaiah, G. M., Segrest, J. P., & Compans, R. W. (1992) *J. Biol. Chem. 267*, 7121−7127.
Stritmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salveson, G. S., & Roses, A. D. (1993a) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1977−1981.
Strittmatter, W. J., Weisgraber, K. H., Huang, D. Y., Dong, L.-M., Salvesen, G. S., Pericak-Vance, M., Schmechel, D., Saunders, A. M., Goldgaber, D., & Roses, A. D. (1993b) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8098−8102.
Taffs, R. E., & Sitkovsky, M. V. (1992) *J. Pharm. Sci. 81*, 37−44.
Taniguchi, T., & Minami, Y. (1993) *Cell 73*, 5−8.
Tosteson, M. T., & Tosteson, D. C. (1981) *Biophsy. J. 36*, 109−116.
Tytler, E. M., Segrest, J. P., Epand, R. M., Nie, S.-Q., Epand, R. F., Mishra, V. K., Venkatachalapathi, Y. V., & Anantharamaiah, G. M. (1993) *J. Biol. Chem. 268*, 22112−22118.
Weisgraber, K. H., Innerarity, T. L., Harder, K. J., Mahley, R. W., Milne, R. W., Marcel, Y. L., & Sparrow, J. T. (1983) *J. Biol. Chem. 258*, 12348−12354.
Weisgraber, K. H., Rall, S. C., Jr., Mahley, R. W., Milne, R. W., Marcel, Y. L., & Sparrow, J. T. (1986) *J. Biol. Chem. 261*, 2068−2076.
Wilson, C., Wardell, M. R., Weisgraber, K. H., Mahley, R. W., & Agard, D. A. (1991) *Science 252*, 1817−1822.
Wisniewski, T., & Frangione, V. (1992) *Neurosci. Lett. 135*, 235−238.
Wisniewski, T., Golabek, A., Matsubara, E., Ghiso, J., & Frangione, B. (1993) *Biochem. Biophys. Res. Commun. 192*, 359−365.
Yankner, B. A., Duffy, L. K., & Kirschner, D. A. (1990) *Science 250*, 279−282.
Ye, S. Q., Reardon, C. A., & Getz, G. S. (1993) *J. Biol. Chem. 268*, 8497−8502.

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