PHYSIOLOGY AND REPRODUCTION

Inhibition of Chicken Adipocyte Differentiation by In Vitro Exposure to Monoclonal Antibodies Against Embryonic Chicken Adipocyte Plasma Membranes

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ABSTRACT Specific monoclonal antibodies (MAb) against adipocyte precursor antigens were developed. These MAb identified adipocyte precursors and reduced their prominence in primary stromal-vascular (SV) cultures by complement-mediated cytotoxicity or by inhibition of differentiation. Binding of antibodies to chicken adipocyte precursors was confirmed by immunofluorescence visual examination following secondary exposure to fluorescein isothiocyanate-conjugated goat anti-mouse IgG. Cross-reaction of MAb with muscle, kidney, liver, fibroblasts, and other cell types not containing lipid droplets was not observed in primary cultures. Adipocyte precursors were obtained from 18-d chick embryo adipose tissue by collagenase digestion to investigate complement-mediated cytotoxicity of preadipocytes. Cultures were maintained in Medium 199 with 5% fetal bovine serum (FBS) for 4 d. Subsequently, Medium 199 supplemented with 10% chicken serum initiated adipocyte differentiation. At Day 5 postinoculation, individual or combinations of MAb were administered to preadipocyte cultures; rabbit complement was added 30 min later. After 1 d of incubation, four of the six individual MAb with complement significantly (P < 0.05) reduced the number of fat cell clusters that developed by 40 to 60%. These MAb in the presence of complement also significantly (P < 0.05) reduced mean cell width and apparent cell area or cell cluster area of lipid-containing cells. Neither MAb nor complement alone reduced fat cell cluster number, cell size, or cluster size. Treatment with pools of two and four MAb decreased the total amount of MAb protein required to reduce fat cell cluster number. Four antibodies, alone or in combination, reduced fat cell cluster development in a complement-dependent manner.

(Key words: antibody, cytotoxicity, differentiation, adipocyte, fat)

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INTRODUCTION

Selection for increased body weight or increased growth rate in chickens improves production efficiency but often yields excessive fat deposition (Cartwright et al. 1986; 1988). Excessive fat deposition affects feed cost, animal health, consumer perception, and marketability of poultry products. Therefore, reduction of fat in poultry is considered desirable.

Anti-adipocyte sera have been used in attempts to reduce body fat in the rabbit (Dulor et al., 1990), chicken (Butterwith et al., 1989), sheep (Nassar and Hu, 1991a), rat (Flint, 1998), and swine (Kestin et al., 1993; Wright and Hausman, 1995; De clerq et al., 1997). Regardless of antiserum specificity, complement-mediated mechanisms were responsible for their cytotoxicity, at least in vitro (Flint et al., 1986; Wright and Hausman, 1995; De clerq et al., 1997), and likely were responsible, in part, for their effect in vivo. Compared to mammalian studies, little research on avian anti-adipocyte antibodies exists. Butterwith et al. (1989) failed to reduce body fat content in chickens because their use of polyclonal antibodies lacked tissue specificity. They reported that chickens administered anti-chicken adipocyte polyclonal antibodies in vivo became unhealthy and developed a green color. This effect was due to a cross-reaction of their polyclonal antibodies with red blood cells.

Surface membranes of living cells possess a wide variety of molecules. These surface molecules mediate cell-recognition events, receptor-ligand interactions, hormonal-signaling events, surrounding structure attachment, and surface homeostasis and transport regulation. Thus, different cell types can display membrane molecules that are charac-

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Abbreviation Key: DME = Dulbecco’s modified Eagle’s/Ham’s F12 medium; FBS = fetal bovine serum; HBSS = Hank’s balanced salt solution; HEPES = N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; MAb = monoclonal antibody; SV = stromal-vascular.
teristic of the cell type and the physiological role of the cell. Relatively few cell surface markers that are expressed preferentially by adipocytes and their precursors have been identified. Primary stromal-vascular (SV) cultures depleted of adipocytes or preadipocytes at a particular stage of development would be a useful model system for establishing conditions or identifying signals that either evoke or permit commitment of adipose lineage cells at a critical point in their developmental history.

When grown in culture, adipocyte precursors proliferate to form a monolayer and then undergo differentiation to become mature adipocytes. Mature adipocytes are characterized by distinct morphological and metabolic changes. These changes include the appearance of specific surface antigens (Plaas et al., 1981; Cryer, 1984), increased expression of differentiation-specific proteins (Cook et al., 1985a,b; Zezulak and Green, 1985), large increases in lipid filling enzyme (Björntorp et al., 1978; Cryer et al., 1987), increased cellular activity of lipogenic enzymes (Coleman et al., 1978), increased activity of lipoprotein lipase (Wise and Green, 1978; Hood, 1982), and increased responsiveness to lipolytic agents (Forest et al., 1981; Gaskins et al., 1989). Expression of mRNA of glycerophosphate dehydrogenase, a 13-kDa lipid-binding protein, and a 28-kDa serine protease-like protein (adipsin) also increase in abundance during adipocyte differentiation (Cook et al., 1985a,b; Zezulak and Green, 1985). Differentiation of adipogenic precursor cells into mature adipocytes occurs by an ordered expression of adipocyte-specific genes regulated by a set of transcription factors that include peroxisome proliferator-activated receptors (Tontonoz et al., 1994) and the CCAAT enhancer binding protein (C/EBP) transcription factor family (Freytag and Geddes, 1992). The unique nature of many of these cell surface molecules was recognized early in the development of immunological techniques for cell identification. Monoclonal antibodies against adipocyte plasma membranes have been developed in swine (Wright and Hausman, 1990a,b; 1995; De clercq et al., 1997) but do not exist for the chicken. This manuscript reports development of monoclonal antibodies against cell surface antigens that are specific to adipocytes in the chicken. The objective of this study was to examine the effect of these anti-adipocyte monoclonal antibodies (MAb) on adipocyte differentiation in SV cultures isolated from chicken embryos.

**MATERIALS AND METHODS**

**Isolation of Chicken Adipocyte Membrane Antigen**

Chicken adipocyte plasma membranes were prepared following the method described by Wright and Hausman (1990a). Femoral and subcutaneous fat depots from 18-d chicken embryos were minced finely with scissors and digested in 20 mL digestion buffer [1 mg/mL collagenase (catalog no. C-2139), 0.1 M N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 0.12 M NaCl, 0.05 M KCl, 5 mM glucose, 1 mM CaCl$_2$, and 1.5% bovine serum albumin]. The digestion mixture was incubated in a shaking water bath at 37 C for 1 h. Digestion buffer without collagenase was used as wash buffer. Room-temperature wash buffer (20 mL) was added to the digestion buffer mixture, and flask contents were filtered simultaneously through sterile nylon screens with nominal pore sizes of 250 and 64 µm to remove aggregates. A floating layer of mature adipocytes was aspirated after centrifugation at 180 × g for 10 min. Cells were washed three additional times in wash buffer by resuspension, centrifugation, and aspiration.

Adipocyte plasma membranes were isolated by cell lysis and centrifugation. Mature adipocytes were mixed vigorously in ice-cold lysis buffer (10 mM Tris-HCl, pH 7.4) for 30 s. Membrane pellets were collected by centrifugation at 12,000×g for 2 min at 4 C. Chicken adipocyte membrane pellets were resuspended with phosphate buffered saline, and total protein concentration was determined spectrophotometrically using a modified Lowry method (Total Protein Kit, catalog no. 690-A).
Antibody Detection and Isolation and Fluorescent Immunoassay

Activated immune cells and ascitic fluids were obtained from mice through protocols approved by Texas A&M University Laboratory Animal Care Committee. BALB/c mice were injected with a plasma membrane mixture containing 60 µg protein (approximately 10 µg per site) in Freund’s complete adjuvant.3 Injections were distributed subcutaneously into inguinal, axial, and tibiotarsal regions for the production of monoclonal antibodies as described in detail by Kearney (1985). Three days after the injection, the injection was repeated in Freund’s incomplete adjuvant. Four more injections of the membrane material in saline alone were administered at 3-d intervals in the concentrations and the sites previously described.

Twenty-four hours after the last injection, lymph nodes draining the subcutaneous areas of injection were used to prepare single-cell suspensions. The lymph nodes of immunized mice were aseptically excised and washed in serum-free RPMI 1640. Lymph nodes were cut and agitated to free cells from connective tissue. Immune lymph node cells were fused with an equal number of SP2/0 myeloma cells by slowly adding 3 to 4 kDa polyethylene glycol (PEG; 50% solution of PEG 4,000 in Ca2+ or Mg2+-free Dulbecco’s phosphate-buffered saline, wt:vol) to the mixture of lymph node and myeloma cells (10^6 cells/mL) in a 1:1 volume ratio. The mixture of cells was incubated in polyethylene glycol solution for 1 to 2 min at 37 C. Serum-free RPMI 1640 medium (20 mL) was slowly added dropwise with gentle swirling over a period of 3 min. The cell solution was centrifuged and then resuspended at a density of 2 x 10^5 cells/mL in RPMI 1640 medium containing antibiotics (100 units penicillin/mL, 0.1 mg streptomycin/mL and 0.25 µg amphotericin B/mL), 20% heat-inactivated FBS, and HAT medium supplements (10^-4 M hypoxanthine, 4 x 10^-7 M aminopterin, and 10^-5 M thymidine at final concentration). Prior to screening, cultures were fed twice at 4-d intervals using the same medium with hypoxanthine and thymidine but lacking aminopterin.

Wells of hybridoma that produced antibodies against adipocytes were detected by screening SV cell cultures containing adipocytes. For initial screening of hybridoma supernatants, cultures were incubated with hybridoma supernatant (initial screening) or 1/500 diluted ascites fluids (all other experiments) for 20 min at 4 C, washed three times with HBSS, and then incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG at 0.1 mg/mL for an additional 20 min. Cultures were rinsed with HBSS and were examined using a Leica fluorescence microscope with Plan 20X and 32X objectives. Exposure times were approximately 30 s for detection of fluorescence. Culture wells containing positive hybrids were cloned twice by limiting dilution to obtain monoclonals (one clone per culture well). These positive clones were retained for future use by cryopreservation in 10% dimethyl sulfoxide freezing medium. Positive clones were further tested for cross-reactivity with other chicken tissue primary cultures (liver, kidney, and muscle) to examine the specificity of MAb against chicken adipocytes.

Monoclonal antibodies specific for adipocytes were obtained by intraperitoneal injection of positive hybridoma clones (10^6 cells/mouse) into previously pristane-primed (0.5 mL; 4 d before inoculation) BALB/c mice to produce ascitic fluids. Purification of MAb from the hybridoma supernate or mouse ascitic fluid was achieved using ammonium sulfate precipitation and then further purified through a Protein G Sepharose column (catalog no. 17-0618-02).5

Cell Culture

Primary cultures of chicken hepatocytes, kidney epithelial cells, muscle cells, and adipose SV cells were used to
examine the specificity of MAb. The liver, kidney, adipose, and muscle tissue samples were obtained from 18-d chicken embryos. All cultures were incubated at 37 C in a humidified atmosphere incubator containing 5% CO₂. The isolation and culture of chicken kidney epithelial cells, muscle cells, hepatocytes, and adipose SV cells were carried out using previously published procedures (Craviso et al., 1987; Duclos et al., 1991; Laurin and Cartwright, 1993; Wu, 1994).

**Adipose SV Cell Culture.** Adipose tissue and adipocyte precursor cultures were obtained from pectoral, femoral, and subcutaneous adipose depots from 18-d chicken embryos. Adipose tissues were excised under a sterile lamellar flow hood and were minced finely with scissors and digested in 20 mL collagenase² (1 mg/mL) digestion buffer. The solution was incubated in a shaking water bath at 37 C for 1 h. Room-temperature wash buffer (20 mL, digestion buffer without enzyme) was added to the digesta. Flask contents were filtered simultaneously through sterile nylon screens with nominal pore sizes of 250 and 64 µm to remove tissue aggregates. The filtered cell suspension was centrifuged at 180 × g for 10 min. The SV fraction pellet was separated from maturing adipocytes contained in the upper layers of supernatant. The SV pellet was resuspended and washed twice with complete culture media (Medium 199 with Earle salts supplemented with 10 mM HEPES, 1 mM sodium pyruvate, 50 mg/L gentamicin sulfate, 2.5 mg/L amphotericin B, and 5% FBS). The cell solution (2 × 10⁵ cells/mL) was plated and grown in complete culture medium between 2 × 10⁴ to 5 × 10⁴ cells/cm² density.

**Hepatocyte Culture.** Hepatocyte cultures were derived from livers of 18-d chicken embryos. Sterilely excised livers were rinsed in ice-cold Ca²⁺ and Mg²⁺-free HBSS and were minced with scissors to 1-mm pieces in 5 mL of HBSS containing 1 mg collagenase. This digestion solution was incubated in a water bath for 1 h. After incubation, the digestion mixture was filtered through a 64-µm nylon screen, and the filtrate was centrifuged for 2 min at 250 × g and 4 C. The supernatant was discarded, and pellets were quickly resuspended in ice-cold HBSS. These washing steps were repeated three times. Upon completion of the final centrifugation, hepatocytes were resuspended, plated, and grown in Dulbecco’s modified Eagle’s/Ham’s F12 medium (DME/F12) supplemented with 10% FBS at

**FIGURE 3.** Chicken muscle cells exposed to monoclonal antibody against chicken adipocyte plasma membranes. Upper micrograph: cultures under visible light. Lower micrograph: cultures of the same view field as upper micrograph but under UV light exposure. Note that the lower micrograph shows no fluorescence following UV excitation. This result contrasted to the fluorescence observed in Figures 1 and 2 (250X magnification).

**FIGURE 4.** Chicken kidney epithelial cells exposed to monoclonal antibody against chicken adipocyte plasma membranes. Upper micrograph: cultures under visible light. Lower micrograph: cultures of the same view field as upper micrograph but under UV light exposure. Note that the lower micrograph shows no fluorescence following UV excitation. This result contrasted to the fluorescence observed in Figures 1 and 2 (250X magnification).
a cell concentration of 2 × 10⁸ viable cells/mL. The plating cell density was between 2 × 10⁵ to 5 × 10⁵ cells/cm².

**Kidney Cell Culture.** Kidney cell cultures were prepared from sterilely excised kidney tissues from 18-d chicken embryos. Kidney tissue was washed in DME, finely minced with scissors, rinsed once with HBSS, and incubated for 15 min at 37 °C in 1 mg/mL collagenase and 0.8 mg/mL hyaluronidase in HBSS. Tissue pieces were gently agitated by pipetting 30 to 40 times over 30 min, and then 10 mL ice-cold HBSS containing 15% FBS was added to the cell mixture. The cells and tubules were collected by centrifugation at 700 × g for 5 min and washed free of red blood cells and other debris by three sequential centrifugation and resuspension steps in HBSS. Cells and tubules were resuspended, plated, and grown at a density between 6 × 10³ to 1.5 × 10⁴ cells/cm² in DME/F12 with 10% FBS and gentamycin (5 µg/mL).

**Muscle Cell Culture.** Muscle cell cultures were derived from embryonic breast muscle tissues. Breast muscle was collected from 18-d chicken embryos under sterile conditions. The tissue was chopped finely in PBS and enzymatically dispersed for 1 h at 37 °C by using trypsin (2.5 mg/mL; added to the solution at 0.25%, wt:vol). DNase I (0.2 mg/mL) was added for the last 5 min of incubation, and tissue fragments were harvested by centrifugation at 1,500 × g for 5 min. The fragments were resuspended in DME containing 10% FBS, penicillin (100 IU/mL), and streptomycin (100 µg/mL) and were triturated through a fine pipette to disperse the cells. An enriched population of myogenic cells was recovered in the supernatant after centrifugation at 700 × g for 1 min. These cells were harvested by centrifugation at 1,500 × g for 5 min and filtered through a 200-µm nylon screen. Cells were plated at a density between 10⁵ to 2.5 × 10⁶ cells/cm² and were grown in DME containing 10% FBS.

**Adipocyte Precursor Cytotoxicity and Inhibition Assay**

**Adipocyte Cultures.** Chicken adipocyte precursor cultures were obtained as previously described. Cells were seeded in 35-mm tissue culture plates at a density of 2 × 10⁴ cells/cm². Basal medium was Medium 199 supplemented with 100 U penicillin/mL, 100 µg gentamycin/mL, and 2.5 µg amphoterin B/mL. All cultures were initiated in basal medium supplemented with 5% FBS. After 4 d, cultures were changed to medium supplemented with 10% chicken serum (differentiation medium). Chicken serum was prepared from serum collected from 14-d-old broiler chickens grown at the Texas A&M University Poultry Research, Teaching and Extension Center. Chicken serum was filtered through a 0.2-µm membrane and frozen until used in the experiments.

**MAb.** Six anti-adipocyte Mab, specifically reactive against chicken adipocytes as described in this report, were used. These MAb were chosen because they were verified by indirect immunofluorescence assay and immunoreactivity in vitro as specific to adipocytes or lipid droplet-containing cells. All 6 MAb are IgG κ isotypes as determined by ELISA with a commercial isotyping kit.

**In Vitro Complement-Dependent Lipid-Containing Cell Loss.** The effects of MAb in the presence of complement were determined in cultures of differentiated and maturing adipocytes. Primary cultures of differentiated and maturing adipocytes without exposure to MAb and complement were used as a negative control in comparisons. Positive controls were exposed to antibodies or complement but not both. Monoclonal antibody and complement exposure treatments consisted of exposure to a single MAb or a combination of antibodies with additional exposure to complement. Treatment exposure was for a period of 24 h from Days 5 to 6 of culture. Each of the six MAb was tested alone and as part of pools of two or four Mab, as indicated in the figure legends and tables. Final concentration of single antibody treatments was 25 µg/mL. When combinations of MAb were exposed to cultures, two cumulative concentrations were used. Two antibody combinations were given at a high cumulative concentration of 20 µg/mL (10 µg/mL each) and a lower cumulative concent-
tration of 2.5 μg/mL (1.25 μg/mL each). Four antibody combinations were given at a high cumulative concentration of 20 μg/mL (5 μg/mL each) and a lower cumulative concentration of 2.5 μg/mL (0.63 μg/mL each). Rabbit complement (catalog no. C-7764) 2 (1/20) was added 30 min after MAb were added to cultures. Cultures were stained for lipid with oil red O to visualize lipid droplets (Hausman, 1981) on Day 7 postinoculation. Cell cluster number, cell length, and cell width were measured by microscopic reticule, and apparent cell area was calculated.

**Statistical Analysis**

Approximately 100 cells per well of six-well culture plates were measured for cell length and width. Apparent cell area was calculated from each cell’s data. Cell cluster numbers were counted in 10 fields of view for each well, constituting approximately 9.6 cm² surface area. Four wells were measured per treatment. Data were subjected to analysis of variance using the general linear model (GLM) procedure of SAS (SAS, 1985). Difference between treatment means was determined by least squares contrast by using the PDIF option of SAS.

**RESULTS AND DISCUSSION**

The primary objective of this study was to produce antibodies that react with antigens specific to chicken adipocytes. These antibodies were then used to reduce adipocyte development in tissue cultures.

**MAb Specificity**

The initial fusion hybrids and the eventual clones were screened using an immunofluorescence technique against adipocytes immediately derived from primary cultures of an embryonic adipose tissue source. Further purification and characterization of the plasma membrane fraction that was used as the immunogen were not necessary, because the adipocyte precursors were used as the detection antigen.

Many MAb that enabled fluorescent labeling of adipocytes were identified. Of these MAb, six (A3E2, C2D10, C4E4, C4D6, D2F5, and D2G3) specifically identified lipid droplet-containing cells (Figure 1 for C4E4 and Figure 2 for C2D10; other data not shown). In those cell cultures containing adipocytes undergoing maturation and lipid filling, immunofluorescence patterns were most apparent in cells with lipid accumulation. Cells containing larger lipid droplets displayed more intense fluorescence than cells with smaller or no lipid droplets.
Two types of fluorescence staining of chicken adipocytes were apparent by microscopic examination. One type of staining was associated with lipid droplets (Figure 2), and the other appeared to diffusely stain the cytoplasm and the lipid droplets (Figure 1). The type of staining and the relative proportions of staining were characteristic of the particular MAb. Clones of D2F5 and C4E4 more diffusely stained adipocytes when compared with A3E2, C2D10, C4D6, and D2G3 characteristics. The basis for these staining differences may be due to different MAb specificities against several adipocyte antigens.

Muscle cells, kidney epithelial cells, hepatocytes, and fibroblasts attendant to adipocyte cultures were used in the immunofluorescence assay to test the tissue specificity of MAb. These MAb did not bind muscle (Figure 3) or kidney epithelial cells (Figure 4). Although these MAb did not fluorescently stain most hepatocytes, lipid-containing cells in embryonic chicken hepatocyte cultures were stained (Figure 5). The cross-reaction of these MAb against different cell types is summarized in Table 1.

In contrast to many mammals, the liver rather than adipose tissue is the major organ of lipogenesis in chickens. Embryonic chicken liver contains a higher percentage of lipid storage cell types than is found in the adult chicken liver because of yolk lipid utilization. Leptin, an adipose-specific protein in mice, rats, and humans (Friedman, 1998; Houseknecht et al., 1998) is also expressed in the liver of chickens (Taouis et al., 1998; Ashwell et al., 1999). Our data suggest that antigens specific to maturing adipocytes are shared by lipid-containing hepatocytes in the chicken. As reported previously (Wright and Hausman, 1991; Nassar and Hu, 1991b; 1992), acquisition of the adipocyte phenotype is accompanied by the expression of specific cell-surface antigens. The specific characteristics of the cell antigens bound by each MAb were not examined.

### MAbs Effects on Adipocyte Development

Four of the six MAb (D2F5, A3E2, C4E4, and D2G3), in combination with complement, significantly reduced (P < 0.05) the fat cell cluster number in cultures when compared with either negative or positive controls (Figures 6 and 7). Neither complement nor MAb treatment alone reduced adipocyte cluster number. One of the MAb (C4E4; Figure 7) caused a small but significant increase (P < 0.05) in the number of adipocyte clusters when compared with both controls. The reason for this effect was unknown. However, antibodies that stimulate adipocyte development exist in porcine adipocyte primary cultures (Wright and Hausman, 1995).

Monoclonal antibodies D2F5, A3E2, C4E4, and D2G3 significantly reduced mean cell area when compared with their respective positive or negative control values (Table 2). This result was not a function of reduced mean cell length. The differences in apparent cell area were caused by significant (P < 0.05) reduction of cell width by MAb (D2F5, A3E2, C4E4, and D2G3) exposure in combination with complement. These MAb also significantly reduced cell cluster number. The color of the culture medium became yellow (more acidic) within 30 min after complement was added to MAb treatments. Increased acidity within such a short period of time suggests cell damage, possibly indicative of cell death. Microscopic examination of cultures stained with trypan blue demonstrated dye-stained cells within 10 to 20 min after complement was added. Dye staining indicates loss of cell viability or death because of the inability of a cell to exclude the dye. The relatively healthy appearance of cells remaining in the treated cultures (fibroblasts) suggested that there was apparently little, if any, damage to the majority of SV cells (Figure 6).

High concentrations of pools of two or four MAb effectively reduced the number of fat cell clusters in adipocyte cultures (Figure 8). We hypothesized that increasing cell surface MAb binding density would amplify the complement-mediated reduction in fat cell cluster number. Increased MAb density was attempted by pooling several MAb. The pooled MAb treatments decreased the number of fat cell clusters to a similar degree at 20% less MAb cumulative concentration as compared with exposure to a single MAb (Figure 7). However, reduction of total MAb concentration to 1/4 of the high dose eliminated the effect on cell cluster number (Figure 8). Similarly, Hausman and Wright (1995) found that whether MAb bound the same antigen or not, they obtained more cell cluster reduction when MAb were used in combinations. Therefore, the ef-
TABLE 1. Primary culture cell specificity of monoclonal antibodies initially derived after exposure to chicken adipocyte plasma membrane antigens

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Monoclonal antibodies¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A3E2</td>
</tr>
<tr>
<td>Adipocyte precursors²</td>
<td>+</td>
</tr>
<tr>
<td>Kidney epithelial cells</td>
<td>–</td>
</tr>
<tr>
<td>Muscle cells</td>
<td>–</td>
</tr>
<tr>
<td>Hepatocytes³</td>
<td>±</td>
</tr>
<tr>
<td>Fibroblasts⁴</td>
<td>–</td>
</tr>
</tbody>
</table>

¹Fluorescently stained cells indicated by +, whereas – indicates absence of staining.
²Adipocyte precursors were defined as fibroblast-like cells with small lipid droplets.
³Embryonic hepatocyte cultures contained a mixture of lipid- and nonlipid-containing cells. Lipid-containing cells rather than nonlipid-containing cells were fluorescently stained in response to exposure to monoclonal antibodies against chicken adipocyte plasma membranes.
⁴Many cells in adipocyte cultures displayed typical fibroblast morphology similar to adipocyte precursors but did not fluorescently stain, nor did they contain lipid droplets.

did not bind chicken muscle, kidney, nonlipid droplet-containing liver cells, or fibroblasts attendant to adipocyte cultures. Development of MAb against chicken plasma membranes with a degree of cell-type specificity was accomplished. This study demonstrated for the first time the isolation of anti-adipocyte MAb specific for lipid droplet-containing cells in chicken primary cultures. Our experiments demonstrated that MAb specific for lipid-containing cells in chickens could be used to identify developing adipocytes. Certain MAb when combined with complement dramatically reduced the number of adipocytes in culture.

ACKNOWLEDGMENT

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TABLE 2. Means of chicken adipocyte cell length and width and apparent cell area after exposure to monoclonal antibody (MAb), or complement, or both in vitro

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cell length (µm)</th>
<th>Cell width (µm)</th>
<th>Apparent cell area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (without MAb and complement)</td>
<td>64.75 ± 4.12</td>
<td>41.75 ± 5.13</td>
<td>2227.5 ± 347.5</td>
</tr>
<tr>
<td>Complement only</td>
<td>65.75 ± 3.15</td>
<td>37.00 ± 4.70</td>
<td>2152.5 ± 235.9</td>
</tr>
<tr>
<td>C2D10 without complement</td>
<td>56.75 ± 4.75</td>
<td>38.75 ± 3.46</td>
<td>1811.3 ± 122.6</td>
</tr>
<tr>
<td>C2D10 with complement</td>
<td>58.00 ± 5.75</td>
<td>32.00 ± 4.19</td>
<td>1845.0 ± 149.8</td>
</tr>
<tr>
<td>D2F5 without complement</td>
<td>56.75 ± 6.82</td>
<td>32.50 ± 3.80</td>
<td>1833.8 ± 109.7</td>
</tr>
<tr>
<td>D2F5 with complement</td>
<td>54.50 ± 4.88</td>
<td>11.50 ± 1.09ᵇ</td>
<td>600.0 ± 54.9ᵇ</td>
</tr>
<tr>
<td>A3E2 without complement</td>
<td>58.75 ± 4.11</td>
<td>33.50 ± 4.85</td>
<td>1958.8 ± 122.8</td>
</tr>
<tr>
<td>A3E2 with complement</td>
<td>58.00 ± 3.25</td>
<td>15.25 ± 1.12ᵇ</td>
<td>858.8 ± 38.2ᵇ</td>
</tr>
<tr>
<td>C4E4 with complement</td>
<td>57.25 ± 3.35</td>
<td>40.75 ± 3.98</td>
<td>2300.0 ± 335.8</td>
</tr>
<tr>
<td>C4E4 with complement</td>
<td>55.00 ± 4.71</td>
<td>12.25 ± 1.28ᵇ</td>
<td>656.3 ± 67.9ᵇ</td>
</tr>
<tr>
<td>D2G3 without complement</td>
<td>60.25 ± 5.76</td>
<td>35.00 ± 3.32</td>
<td>2085.0 ± 136.5</td>
</tr>
<tr>
<td>D2G3 with complement</td>
<td>55.25 ± 6.53</td>
<td>14.00 ± 1.24ᵇ</td>
<td>743.8 ± 70.3ᵇ</td>
</tr>
<tr>
<td>C4D6 without complement</td>
<td>58.75 ± 5.36</td>
<td>35.25 ± 3.96</td>
<td>1861.3 ± 124.5</td>
</tr>
<tr>
<td>C4D6 with complement</td>
<td>61.00 ± 7.67</td>
<td>32.25 ± 5.00</td>
<td>1853.8 ± 115.4</td>
</tr>
</tbody>
</table>

¹Cell length and width were measured by microscopic reticule (µm) 1 d after administration of individual MAb (25 µm/ml final dilution); complement was added 30 min after MAb administration. Apparent cell area is defined as cell length x width (µm²).
²Significant differences (P < 0.05) from negative control group (without MAb and complement).
³Significant differences within MAb treatment (with vs. without complement).
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