รายงานวิจัยฉบับสมบูรณ์

โครงการฤทธิ์ต้านแบคทีเรียของ high-density lipoproteins ในภาวะตอบสนอง acute-phase

(Antimicrobial activity of high-density lipoproteins (HDL) during the acute-phase response)

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คณะผู้วิจัย	สังกัด
1. นายแพทย์ วีรพันธุ์ โชควิฑูรกิจ	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
2. รองศาสตราจารย์ ดร. สุทธิลักษณ์ ปทุมราช	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
3. นางสาวเปรมทิพย์ ทวีรติธรรม	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
4. นางสาววรกมล แน่นอุดร	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สนับสนุนโดยทบวงมหาวิทยาลัย และ สำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย ทบวงฯ และสกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

บทคัดย่อ

HDL (High-density lipoprotein) เป็นกลุ่มของ lipoprotein ซึ่งมีหน้าที่สำคัญในการลำเลียงคอเลสเตอรอลส่วนเกินออกจากเซลล์และป้องกันปฏิกิริยา oxidation ของ LDL ในขณะที่ร่างกายมีการอักเสบติดเชื้อ HDL มีส่วนประกอบและหน้าที่เปลี่ยนแปลงไป เมื่อร่างกายเกิดการติดเชื้อแบคทีเรีย พบว่า endotoxin หรือ lipopolysaccharide (LPS) ของเชื้อแบคทีเรียกรัมลบ และ lipoteichoic acid (LTA) ของเชื้อแบคทีเรียกรัมบวก สามารถกระตุ้นร่างกายให้เกิดการหลั่ง cytokines ต่างๆและมีผลก่อให้เกิด septic shock ในคนไข้ติดเชื้อดังกล่าวได้ การศึกษาที่ผ่านมา พบว่า HDL ยังมีความสำคัญในระบบภูมิคุ้มกัน โดย HDL สามารถจับกับ LPS และ LTA รวมทั้งลดพิษที่เกิดขึ้นกับร่างกาย ทำให้เซลล์หลั่ง cytokines ออกมาน้อยลงได้ โครงการวิจัยนี้ทำขึ้นเพื่อศึกษาว่า HDL มีบทบาทอย่างไรในการเจริญเติบโตของแบคทีเรีย HDL ถูกปั่นแยกจากซีรัมของหนูทดลองปกติ และหนูทดลองที่มีการกระตุ้นด้วย LPS จากนั้นนำ HDL มาทดสอบว่ามีฤทธิ์ในการยับยั้งการเจริญเติบโตของแบคทีเรียหรือไม่ ผลการทดลองพบว่า ทั้ง HDL ปกติและ HDL ที่เกิดขึ้นเมื่อร่างกายถูกกระตุ้น ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียได้ทั้งเชื้อกรัมบวกและเชื้อกรัมลบ ที่ความเข้มข้นต่างๆกันและที่ช่วงเวลาต่างๆกัน อย่างไรก็ตาม พบว่า apo A-I ซึ่งเป็นโปรตีนหลักของ HDL มีฤทธิ์ยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียกรัมกรัมลบ คือ Escherichia coli ได้ แต่ไม่มีฤทธิ์ในการยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียกรัมกรัมบวก Staphylococcus epidermidis ผลการศึกษานี้ แสดงถึงบทบาทของ apo A-I ในกระบวนการของร่างกายเมื่อมีการติดเชื้อแบคทีเรียกรัมลบ และควรที่จะมีการศึกษาต่อถึงประโยชน์ของ apo A-I ในสัตว์ทดลองต่อไป

Abstract

HDL is a group of lipoproteins which play an important role in removing excess cholesterol from cells and in preventing oxidation of LDL. During infection and inflammation, there are changes in the composition and function of HDL. During bacterial infection, endotoxin or lipopolysaccharide (LPS) from gram-negative bacteria and lipoteichoic acid (LTA) from gram-positive bacteria can induce cytokine release causing septic shock in infected patients. Previous studies have shown that HDL, as part of innate immunity, can bind both LPS and LTA and ameliorate their toxic effects, resulting in decreased cytokine release. This study was performed to examine the effect of HDL on the growth of bacteria. HDL was isolated from sera of normal hamsters and LPS-injected hamsters, and subsequently incubated with bacteria to assess bacterial growth. The results show that both normal HDL and acute-phase HDL (from LPS-injected animals) could not inhibit the growth of either gram-negative or gram-positive bacteria. Similarly, apo HDL, the protein portion of HDL, could not inhibit the growth, whereas apolipoprotein A-I (apo A-I), the main protein of HDL, significantly inhibited the growth of gram-negative Escherichia coli, but not gram-positive Staphylococcus epidermidis. Our result suggests an important role of apo A-I in the host defense against gram-negative bacterial infection. Further study in in vivo models is warranted.

Executive Summary

ทุนพัฒนาศักยภาพในการทำวิจัยของอาจารย์รุ่นใหม่

ฤทธิ์ต้านแบคทีเรียของ high-density lipoproteins ในภาวะตอบสนอง acute-phase

วีรพันธุ์ โชควิฑูรกิจ

1. ความสำคัญและที่มาของปัญหา

โรคติดเชื้อจากแบคทีเรียเป็นโรคที่พบบ่อย และเป็นสาเหตุสำคัญอันดับต้นๆของการตายของผู้ป่วยในโรงพยาบาล ยาปฏิชีวนะที่ใช้กันอยู่ในปัจจุบัน สามารถรักษาการติดเชื้อจากแบคทีเรียได้ส่วนหนึ่ง แต่เมื่อใช้ในระยะยาว เชื้อแบคทีเรียจะเกิดการดื้อยา ทำให้การรักษาด้วยยาปฏิชีวนะนั้นไม่ได้ผล การคิดค้นหายาปฏิชีวนะกลุ่มใหม่หรือสารใหม่ๆที่มีฤทธิ์ยับยั้งการเจริญเติบโตของแบคทีเรีย จึงมีความสำคัญอย่างยิ่งในการรักษาการติดเชื้อดังกล่าว

ในระหว่างที่ร่างกายมีการติดเชื้อแบคทีเรีย ร่างกายมีการตอบสนองที่เรียกรวมว่า acute-phase response ซึ่งช่วยป้องกันร่างกายไม่ให้มีการบาดเจ็บเพิ่มขึ้น และช่วยในกระบวนการซ่อมแซมของร่างกาย [1] ปฏิกิริยาตอบสนองนี้เห็นได้จากการที่มีการเพิ่มขึ้นของระดับโปรตีนที่เป็น acute-phase reactant ในเลือด เช่น C-reactive protein, fibrinogen และ serum amyloid A ในขณะเดียวกัน ระดับไขมันในเลือดมีการเปลี่ยนแปลงในหลายๆด้าน เช่น มีการเพิ่มขึ้นของระดับไตรกลีเซอไรด์ (triglyceride) และ การลดลงของคอเลสเตอรอล (cholesterol) [2] การเปลี่ยนแปลงทางไขมันดังกล่าวจะมีผลดีต่อร่างกายโดยรวมอย่างไร ยังไม่เป็นที่ทราบอย่างแน่ชัด

ไขมันชนิดต่างๆที่อยู่ในเลือดนั้น เกาะรวมกลุ่มอยู่กับโปรตีนในรูปของ lipoproteins ซึ่งเป็นอนุภาคขนาดเล็ก Lipoproteins สามารถแบ่งออกได้เป็นหลายประเภทตามความหนาแน่น (density) และโปรตีนที่เป็นส่วนประกอบหลัก Very low-density lipoprotein (VLDL) และ Low-density lipoprotein (LDL) ซึ่งมี apolipoprotein B-100 เป็นโปรตีนสำคัญ ทำหน้าที่ลำเลียงกรดไขมันและคอเลสเตอรอลไปยังเนื้อเยื่อ เพื่อใช้เป็นพลังงานและส่วนประกอบของผนังเซลล์ สำหรับ High-density lipoproteins หรือ HDL นั้น มีขนาดเล็กกว่า แต่มีความหนาแน่นมากกว่า VLDL และ LDL ไขมันที่เป็นส่วนประกอบหลักของ HDL คือ คอเลสเตอรอลและฟอสโฟลิปิด สำหรับไตรกลีเซอไรด์นั้นมีเป็นส่วนน้อย โปรตีนที่พบบน HDL มีมากกว่า 30 ชนิด ซึ่งโปรตีนแต่ละชนิดมีหน้าที่ต่างๆกันไป โปรตีนที่เป็นส่วนประกอบสำคัญและเป็นโครงสร้างหลักของ HDL คือ apolipoprotein A-I หรือ apo A-I

การศึกษาทางระบาดวิทยา และการวิจัยทางคลินิก แสดงให้เห็นว่า ระดับของ lipoproteins ชนิดต่างๆ มีความสัมพันธ์กับการเกิดโรคหลอดเลือดแดงแข็ง (atherosclerosis) [3] โดยที่ ระดับ LDL ที่สูง

และระดับ HDL ที่ต่ำเป็นปัจจัยเสี่ยงที่สำคัญต่อการเกิดโรคหลอดเลือดแดงแข็ง ผลของการศึกษาวิจัยสนับสนุนสมมติฐานที่เชื่อว่า HDL ป้องกันโรคหลอดเลือดแดงแข็งได้ โดยการนำคอเลสเตอรอลส่วนเกินออกจากเซลล์เพื่อขับออกจากร่างกาย [4]

ในระหว่างที่ร่างกายมีการติดเชื้อ พบว่ามีการเพิ่มขึ้นของระดับไตรกลีเซอไรด์ จากการเพิ่มการสร้าง VLDL จากตับ และ มีการลดลงของระดับ LDL และ HDL cholesterol [2] นอกจากนี้ส่วนประกอบของ lipoproteins ยังมีการเปลี่ยนแปลงไปทั้งในส่วนของไขมันและโปรตีน การเปลี่ยนแปลงเหล่านี้ยังผลให้หน้าที่การทำงานของ lipoproteins เปลี่ยนแปลงไปด้วย

งานวิจัยของข้าพเจ้าในช่วง 3-4 ปีที่ผ่านมา ทำการศึกษาเกี่ยวกับการเปลี่ยนแปลงของ HDL ในระหว่างที่ร่างกายมีปฏิกิริยาตอบสนองต่อการติดเชื้อ โดยฉีด endotoxin เข้าไปในหนูทดลอง (Syrian hamsters) เพื่อกระตุ้นให้เกิด acute-phase response แล้วทำการปั่นแยก HDL ออกมา งานวิจัยดังกล่าวแสดงให้เห็นว่า HDL ซึ่งเกิดขึ้นในระหว่างที่ร่างกายมีปฏิกิริยาตอบสนองต่อการติดเชื้อ ที่เรียกกันว่า acute-phase HDL นั้น มีส่วนประกอบที่แตกต่างจาก HDL ปกติ ทั้งในด้านของไขมันและโปรตีน การเปลี่ยนแปลงในส่วนประกอบของ acute-phase HDL นี้ ยังผลให้หน้าที่การทำงานของ acute-phase HDL แตกต่างไปจาก HDL ปกติด้วย [5] ดังจะเห็นได้จากการที่ acute-phase HDL มีความบกพร่องในการลำเลียงคอเลสเตอรอลออกมาภายนอกเซลล์ อันเนื่องจากการลดลงของเอนไซม์ lecithin:cholesterol acyltransferase ใน acute-phase HDL [5] การเปลี่ยนแปลงนี้อาจมีผลต่อการเกิดโรคหลอดเลือดแดงแข็งในระยะยาว อย่างไรก็ตาม การเปลี่ยนแปลงของ HDL ในระหว่างที่ร่างกายมีการติดเชื้อ จะมีผลต่อร่างกายในด้านอื่นๆอย่างไร ยังไม่เป็นที่ทราบแน่ชัด

นอกเหนือไปจากหน้าที่ของ HDL ในการลำเลียงคอเลสเตอรอลส่วนเกินออกจากเซลล์แล้ว HDL ยังมีคุณสมบัติอื่นๆที่บ่งชี้ว่ามีความสำคัญในระบบภูมิคุ้มกันสืบทอด (innate immunity) ด้วย การศึกษาวิจัยทั้งนอกร่างกาย (in vitro) และในสัตว์ทดลอง พบว่า HDL มีบทบาทในการจับกับ endotoxin (หรือ lipopolysaccharide-LPS) จากเชื้อแบคทีเรียกรัมลบ และ lipoteichoic acid (LTA) ของเชื้อแบคทีเรียกรัมบวก รวมทั้ง สามารถลดพิษของทั้ง endotoxin และ lipoteichoic acid ได้ [6-18] การศึกษาเพิ่มเติม พบว่า endotoxin ที่จับอยู่กับ HDL นี้ มีฤทธิ์ลดลงกว่า endotoxin ในภาวะอิสระ [6-16] การใช้ HDL สังเคราะห์ในผู้ป่วย endotoxemia พบว่าสามารถลดการหลั่ง cytokines และลดอาการของผู้ป่วยได้เช่นกัน [19]

นอกจากผลของ HDL ที่มีต่อ endotoxin แล้ว การศึกษาจากประเทศญี่ปุ่น พบว่าในภาวะปกติ HDL สามารถยับยั้งการเจริญเติบโตของแบคทีเรีย Staphylococcus epidermidis ภายนอกร่างกายได้ [20] หลังจากที่มีการตีพิมพ์ผลงานดังกล่าว ยังไม่มีการศึกษาเพิ่มเติมว่า HDL มีฤทธิ์ต่อแบคทีเรียชนิดอื่นๆที่

ก่อให้เกิดโรคได้หรือไม่ รวมทั้งยังไม่มีการศึกษาว่าส่วนประกอบใดของ HDL ที่ทำหน้าที่ยับยั้งการเจริญเติบโตของแบคทีเรีย โครงการวิจัยนี้ได้ทำขึ้นเพื่อศึกษาฤทธิ์ของ HDL ปกติและ acute-phase HDL ในการยับยั้งการเจริญเติบโตของแบคทีเรียชนิดต่างๆทั้งกรัมบวก และกรัมลบ รวมทั้งจะได้ทำการศึกษาเพิ่มเติมเพื่อค้นหาส่วนประกอบของ HDL ซึ่งเป็นกลไกในการออกฤทธิ์ดังกล่าว สำหรับเชื้อแบคทีเรียที่ใช้ ประกอบไปด้วยเชื้อแบคทีเรียกรัมบวก เช่น Staphylococcus epidermidis และ Staphylococcus aureus และ เชื้อแบคทีเรียกรัมลบ เช่น Escherichia coli และ Pseudomonas aeruginosa เชื้อแบคทีเรียกรัมลบเหล่านี้เป็นสาเหตุสำคัญของการติดเชื้อแบคทีเรียในเลือด (septicemia) และ พบได้บ่อยในทางคลินิก

โครงการศึกษาวิจัยที่เสนอมานี้ จะก่อให้เกิดความรู้ใหม่และความเข้าใจในบทบาทของ HDL ในระบบภูมิคุ้มกันสืบทอด และจะเป็นพื้นฐานในการศึกษาวิจัยต่อเพื่อค้นคว้าและดัดแปลงหาส่วนประกอบของ HDL ที่มีฤทธิ์ยับยั้งการเติบโตของแบคทีเรีย อันจะก่อให้เกิดประโยชน์ในการนำความรู้มาประยุกต์ใช้ในการออกแบบหาสารที่สามารถรักษาการติดเชื้อแบคทีเรียต่อไป

2. วัตถุประสงค์

วัตถุประสงค์ที่ 1 : ศึกษาเปรียบเทียบฤทธิ์ของ acute-phase HDL กับ HDL ในภาวะปกติ ในการยับยั้งการเจริญเติบโตของแบคทีเรียชนิดต่างๆ ทั้งกรัมบวก และ กรัมลบ

วัตถุประสงค์ที่ 2 : ศึกษาหาส่วนประกอบของ HDL ที่มีฤทธิ์ในการยับยั้งการเจริญเติบโตของแบคทีเรีย

3. ระเบียบวิธีการวิจัย

<u>สารเคมีที่ใช้</u>

Endotoxin (Lipopolysaccharide) จาก Escherichia coli serotype 055:B5 สั่งมาจากบริษัท Sigma ประเทศสหรัฐอเมริกา แบคทีเรียกรัมบวก Staphylococcus epidermidis, Staphylococcus aureus และแบคทีเรียกรัมลบ Escherichia coli และ Pseudomonas aeruginosa สั่งมาจากกรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข อาหารเลี้ยงเชื้อสั่งจากบริษัท Sanofi ประเทศฝรั่งเศส และบริษัท Oxoid ประเทศอังกฤษ การวัดความเข้มข้นของโปรตีน ใช้วิธี modified Lowry assay สารเคมีอื่นๆ ที่ใช้ในการทดลองสั่งมาจากบริษัท Asia Pacific Specialty Chemicals ประเทศออสเตรเลีย

<u>สัตว์ทดลอง</u>

สัตว์ทดลองที่ใช้สำหรับแยก HDL คือหนู Syrian hamsters เนื่องจากการศึกษาที่ผ่านมา พบว่าเมตะบอลิสมของ HDL ของ Syrian hamster ใกล้เคียงกับของคน และการเปลี่ยนแปลงของ HDL ที่

เกิดขึ้นจากการกระตุ้น acute-phase response ด้วย endotoxin มีการศึกษาอย่างกว้างขวาง [21-22]
โครงการวิจัยนี้ ได้รับการอนุมัติจากคณะกรรมการจริยธรรม คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัยแล้ว

Syrian hamsters เพศเมีย อายุ 6-12 สัปดาห์ สั่งมาจากศูนย์สัตว์ทดลอง ศาลายา และเลี้ยงด้วยอาหารและน้ำตามปกติ อย่างน้อย 1 สัปดาห์ก่อนการทดลอง หนูกลุ่มหนึ่งได้รับการฉีดด้วย endotoxin ทางหน้าท้อง (intraperitoneal) ในขนาด 100 ไมโครกรัม/100 กรัมของน้ำหนักตัว ส่วนหนูอีกกลุ่มหนึ่งได้รับการฉีดด้วยน้ำเกลือปราศจากเชื้อ (sterile normal saline solution) หลังจากการฉีดดังกล่าว 16 ชั่วโมง มีการเก็บเลือดของหนูทดลองอย่างปราศจากเชื้อ เพื่อให้ได้ซีรั่ม

<u>การปั่นแยก HDL</u>

Acute-phase HDL และ HDL ปกติ ถูกปั่นแยกโดยวิธี ultracentrifugation จากซีรั่มของหนูทดลองซึ่งได้รับการฉีด endotoxin หรือ น้ำเกลือปราศจากเชื้อ ตามลำดับ Potassium bromide (KBr) ที่ความเข้มข้นต่างๆ ใช้เป็นตัวปรับให้ได้ความหนาแน่นของสารละลายในการปั่นแยกตามต้องการ โดยมีการปั่นแยกเอา VLDL และ LDL ออกจากซีรั่มออกไปก่อน จากนั้น ในการปั่นแยก HDL ใช้เครื่อง L8-70M ultracentrifuge ของบริษัท Beckman โดยปั่นที่ 100,000 g ที่อุณหภูมิ 10 องศาเซลเซียส เป็นเวลา 48 ชั่วโมง HDL ที่ได้ถูกนำมาเปลี่ยนสารละลาย (dialysis) ในน้ำเกลือปราศจากเชื้อที่ประกอบด้วย 0.01% EDTA ก่อนที่จะนำมาผ่าน filter ขนาด 0.45 ไมครอนและใช้ในการทดลองภายใน 2 สัปดาห์ ระหว่างการปั่นแยก HDL ได้ใช้วิธีการที่ปราศจากเชื้อ เพื่อไม่ให้มีการปนเปื้อนของแบคทีเรียหรือ endotoxin ใน HDL ดังกล่าว โดยสารเคมีหรือวัสดุที่ใช้ในการปั่นแยก HDL ถูกทำให้ปราศจากเชื้อด้วย autoclave ถ้าไม่ได้ในรูปที่ปราศจากเชื้อมาก่อน[23]

<u>การสกัด apoHDL</u>

Apo HDL คือ HDL ที่มีเฉพาะโปรตีน แต่ไม่มีไขมันเป็นส่วนประกอบ การสกัด apo HDL ทำได้โดยใช้ ethanol และ ether [24]

<u>การสกัด apo A-I</u>

Apo A-I เป็นโปรตีนหลักของ HDL ในการสกัด apo A-I ออกมาจาก HDL นั้น ทำโดยใช้ HDL ที่แยกได้มาผ่าน SDS gel electrophoresis แล้วย้อมด้วย copper staining เพื่อให้ได้ apo A-I band จากนั้นจึงตัดเอา apo A-I band ออกมา หั่น gel เป็นชิ้นเล็กๆ และนำมาแยกโปรตีน apo A-I ออกจาก gel ด้วยวิธี electroelution ด้วยเครื่อง electroeluter ของบริษัท Bio-Rad จากนั้น apo A-I ที่ได้ถูกนำมาละลายใน 1X phosphate buffered saline และวัดความเข้มข้น [25]

<u>การทดลองเกี่ยวกับแบคทีเรีย</u>

แบคทีเรียที่ได้มา ถูกนำมาเลี้ยงเป็น stock และเก็บไว้ที่ -80 องศาเซลเซียส ในแต่ละการทดลองจะนำแบคทีเรียที่เก็บไว้นี้มาทำการทดลอง เพื่อให้ได้แบคทีเรียที่มีคุณสมบัติเหมือนกันทุกการทดลอง

HDL, apo HDL หรือ apo A-I ที่ความเข้มข้นต่างๆ จะถูกนำไป incubate กับ liquid cultures ของเชื้อแบคทีเรีย การเจริญเติบโตของเชื้อแบคทีเรียจะถูกประเมินที่ระยะเวลาต่างๆกัน ตั้งแต่ 0 ถึง 24 ชั่วโมง การประเมินการเจริญเติบโตของเชื้อแบคทีเรียนั้น กระทำโดยการเจือจางเชื้อและเพาะลงในจานเพาะเชื้อ จำนวน colony ของแบคทีเรียที่ขึ้นจะบ่งถึงจำนวนของเชื้อแบคทีเรียที่มีใน liquid cultures

4. แผนการดำเนินงานวิจัยตลอดโครงการในแต่ละช่วง 6 เดือน

ในช่วง 6 เดือนแรก ได้ดำเนินการพัฒนาวิธีการทดลองทางห้องปฏิบัติการเพื่อปรับสภาพการทดลองให้เหมาะสม ระหว่างนั้นได้สั่งสัตว์ทดลอง โดยใช้สัตว์ทดลองประมาณ 6 ชุดๆละ 12 ตัว ในระยะเวลา 2 ปีที่ทำการวิจัย

ปีที่ 1 (6 เดือนหลัง)

สัตว์ทดลองชุดที่ 1 และ 2: ทำการศึกษา dose response และ time course ของการยับยั้งการเจริญเติบโตของแบคทีเรียของ HDL ทั้ง Acute-phase HDL และ HDL ปกติ

สัตว์ทดลองชุดที่ 3 และ 4: ทำการศึกษาเปรียบเทียบการยับยั้งการเจริญเติบโตของแบคทีเรียที่ก่อให้เกิดโรคชนิดต่างๆ ทั้งกรัมบวก และกรัมลบ โดย Acute-phase HDL และ HDL ปกติ

ปีที่ 2

สัตว์ทดลองชุดที่ 5: เปรียบเทียบระหว่าง HDL กับ apo HDL (HDL ที่สกัดเอาไขมันออก)

สัตว์ทดลองชุดที่ 6: ทำการศึกษา dose response และ time course ของการยับยั้งการเจริญเติบโตของแบคทีเรียของ apo A-I

5. ผลงาน/หัวข้อเรื่องที่คาดว่าจะตีพิมพ์ในวารสารวิชาการระดับนานาชาติในแต่ละปี

ปีที่ 1: ชื่อเรื่องที่ตีพิมพ์ Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host

ชื่อวารสารที่ตีพิมพ์ Journal of lipid research (Impact factor 4.139)

ปีที่ 2: ชื่อเรื่องที่คาดว่าจะตีพิมพ์ Antibacterial activity of native and recontituted high-density lipoprotein: modulation by types of phospholipids and apolipoproteins

ชื่อวารสารที่คาดว่าจะตีพิมพ์ Journal of lipid research (Impact factor 4.139), Atherosclerosis (Impact factor 3.469), หรือ Lipids (Impact factor 2.117)

6. งบประมาณโครงการ

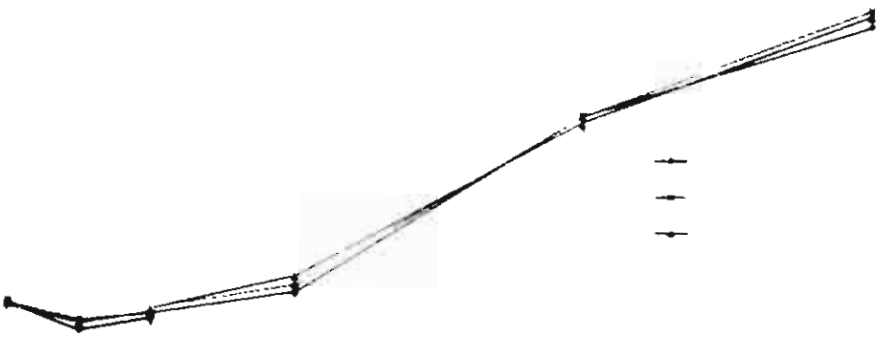
	ปีที่ 1	ปีที่ 2	รวม
1. หมวดค่าตอบแทน			
- ค่าตอบแทนหัวหน้าโครงการ	120,000	120,000	240,000
2. **หมวดค่าวัสดุ**			
- ค่าวัสดุสารเคมี	55,000	67,000	122,000
- ค่าชุดวัดระดับไขมันและโปรตีน	20,000	20,000	40,000
- ค่าสัตว์ทดลอง	24,000	12,000	36,000
- ค่าเชื้อแบคทีเรีย	2,000	2,000	4,000
- ค่าหลอดและจานเพาะเชื้อ	3,000	3,000	6,000
- ค่าสารอาหารเพาะเชื้อ	10,000	10,000	20,000
3. หมวดค่าใช้สอย			
- ค่าจ้างเหมาบริการ	6,000	6,000	12,000
รวมงบประมาณโครงการ	240,000	240,000	480,000

ผลการทดลอง

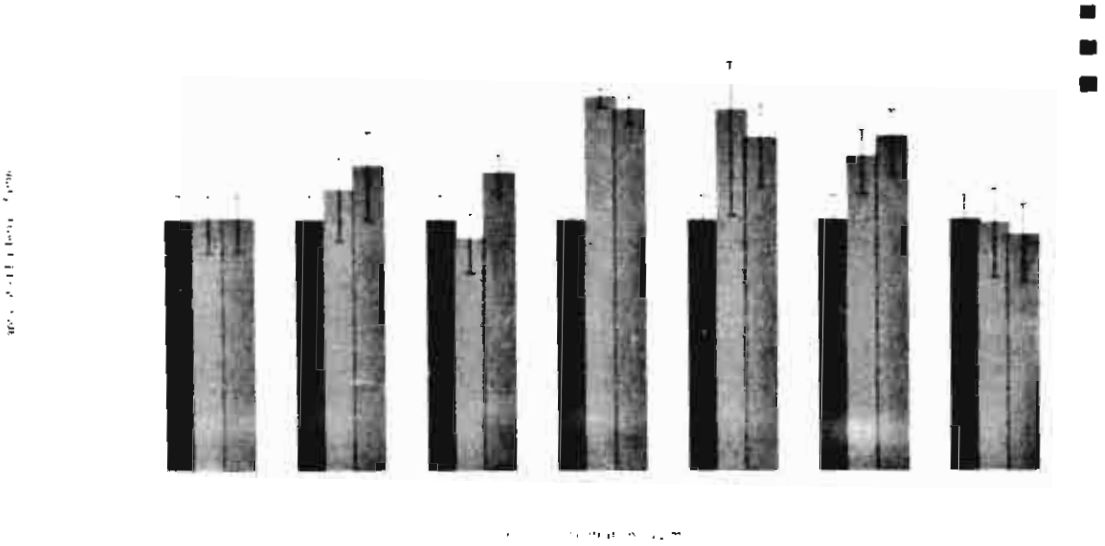
HDL กับการเจริญเติบโตของเชื้อแบคทีเรียกรัมลบ

ผลการทดลองแสดงว่า HDL ปกติที่ความเข้มข้นต่างๆ ตั้งแต่ 0- 1,670 ไมโครกรัม มิลลิลิตร ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรีย Escherichia coli ได้อย่างมีนัยสำคัญ ที่ช่วงเวลาต่างๆกัน ตั้งแต่ 0.5, 1, 2, 4, 6 และ 24 ชั่วโมง ในทางตรงกันข้าม acute-phase HDL ที่ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรีย Escherichia coli ได้เช่นเดียวกับรูปที่ 1 และ 2) สามารถยับยั้งความเข้มข้นของ HDL ที่ใช้นี้อยู่ในช่วง physiologic range ที่จะพบได้ใน interstitial fluid และที่อยู่ในกระแสเลือด

ในการทดลอง Escherichia coli ดังกล่าวได้ทำการทดลองในเชื้อแบคทีเรียกรัมลบอีกชนิดหนึ่ง คือ Pseudomonas aeruginosa ผลการทดลองที่ได้ไม่ต่างจากการทดลอง Escherichia coli ดังแสดงในรูป



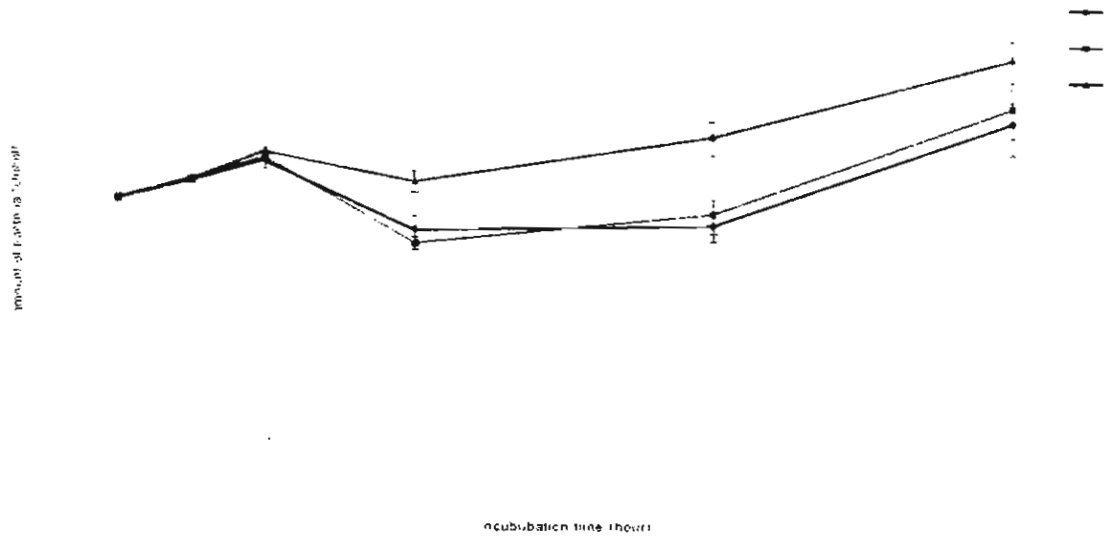
รูปที่ 1 Effect of HDL (500ug/ml) on the growth of E.coli at various timepoints



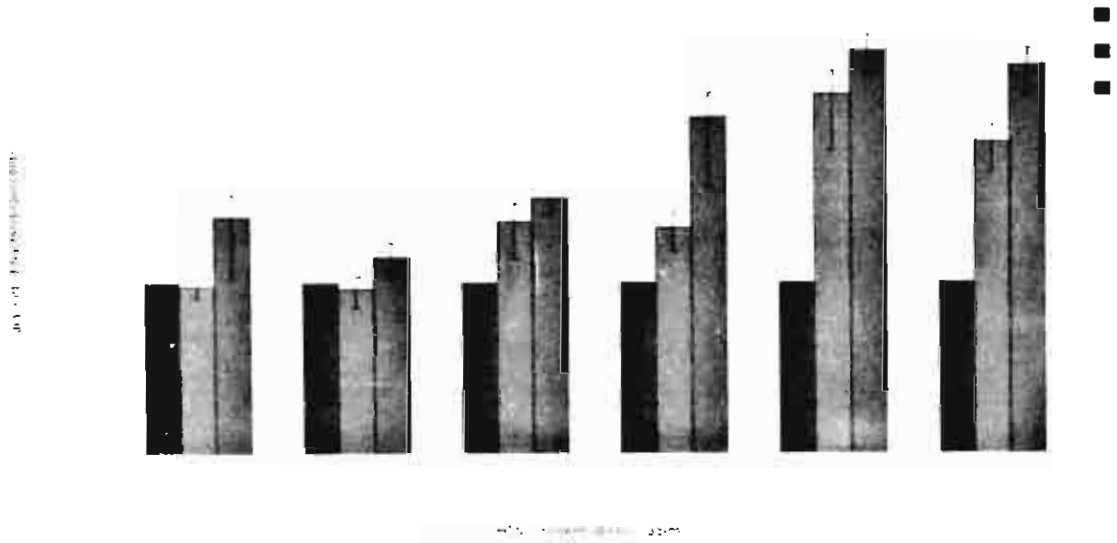
รูปที่ 2. Effect of [illegible] concentrations of HDL on E. coli growth at incubation time [illegible] h

HDL กับการเจริญเติบโตของเชื้อแบคทีเรียกรัมบวก

ผลการทดลองในเชื้อแบคทีเรียกรัมบวก ทั้ง Staphylococcus epidermidis และ Staphylococcus aureus พบว่าผลที่ได้คล้ายคลึงกับผลที่ได้จากเชื้อแบคทีเรียกรัมลบ นั่นคือ ทั้ง HDL ปกติ และ acute-phase HDL ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียดังกล่าวได้ ในช่วงความเข้มข้นของ HDL ต่างกัน และในช่วงเวลาต่างกัน (รูปที่ 3 และ 4)



รูปที่ 3. Effect of 200ug/ml protein of HDL on S epidermidis growth at various timepoints



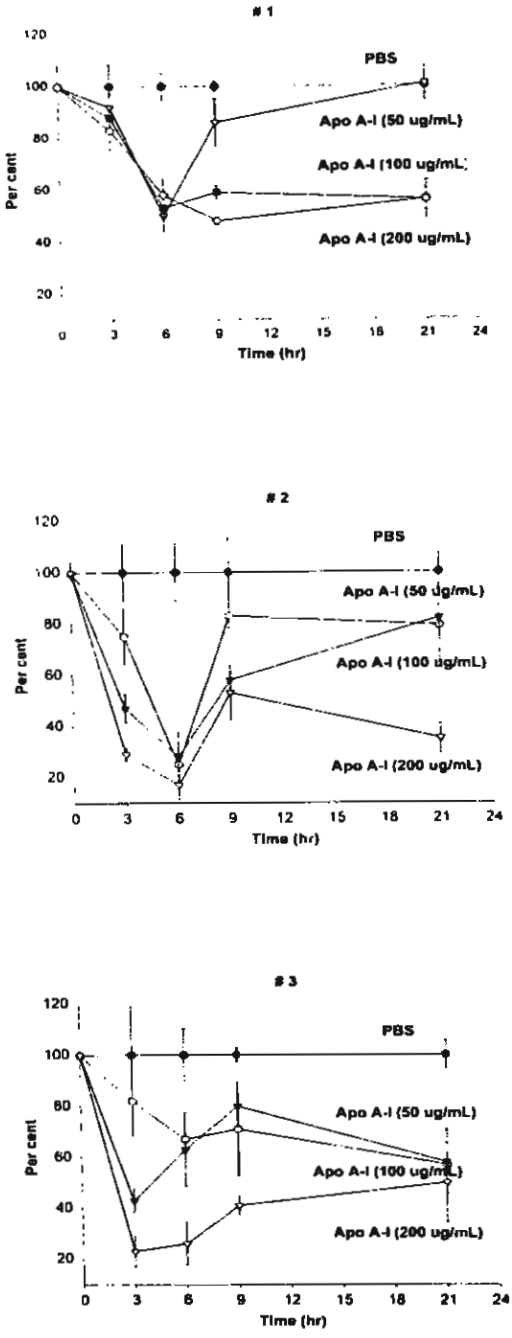
รูปที่ 4 Effect of various concentrations of HDL on S epidermidis growth

Apo HDL กับการเจริญเติบโตของเชื้อแบคทีเรีย

เนื่องจาก HDL ประกอบด้วยส่วนที่เป็นไขมันและโปรตีนรวมกัน การทดลองพบว่า HDL ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียได้ อาจเป็นจากการที่เชื้อแบคทีเรียนำไขมันใน HDL ไปใช้ ซึ่งมีรายงานว่าสารอาหารจากไขมันชนิดต่าง ๆ โดยเฉพาะอย่างยิ่งจากไตรกลีเซอไรด์ สามารถส่งเสริมการเติบโตของเชื้อแบคทีเรียได้ [ ] จึงมีการสกัดเอาส่วนที่เป็นไขมันของ HDL ออก เพื่อให้เหลือเฉพาะส่วนที่เป็นโปรตีนที่เรียกว่า apo HDL แล้วนำมาทดสอบ อย่างไรก็ตาม พบว่า apo HDL ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียทั้งกรัมลบและกรัมบวกได้เช่นกัน (data not shown)

Apo A-I กับการเจริญเติบโตของเชื้อแบคทีเรีย

เพื่อศึกษาว่า apo A-I ซึ่งเป็นโปรตีนหลักของ HDL มีฤทธิ์ในการยับยั้งการเจริญเติบโตของแบคทีเรียหรือไม่ ได้มีการแยก apo A-I ออกมาจาก HDL มาทำการศึกษา พบว่า apo A-I ที่ความเข้มข้น 100 – 200 ไมโครกรัม/มิลลิลิตร สามารถยับยั้งการเจริญเติบโตของแบคทีเรียกรัมลบ Escherichia coli ได้อย่างมีนัยสำคัญที่ 6 และ 9 ชั่วโมง (รูปที่ 5) แต่ไม่มีผลชัดเจนต่อการเจริญเติบโตของแบคทีเรียกรัมบวก Staphylococcus epidermidis (data not shown)



รูปที่ 5. Effect of apo A-I on the growth of Escherichia coli at various time points (These 3 graphs represent 3 different experiments)

วิจารณ์ผล

ผลที่ได้จากการศึกษานี้ พบว่า HDL ทั้ง HDL ปกติ และ acute-phase HDL ไม่ได้มีผลในการยับยั้งการเจริญเติบโตของแบคทีเรียทั้งกรัมลบและกรัมบวก ซึ่งผลดังกล่าวนี้แตกต่างจากผลการศึกษาของ Tada et al. ที่พบว่า HDL ปกติ สามารถยับยั้งการเจริญเติบโตของ Staphylococcus epidermidis ได้ [20] สาเหตุของความแตกต่างนี้ คาดว่าเกิดจากการที่ Tada et al. ใช้ phosphate-buffered saline (PBS) ในการเพาะเชื้อ ในขณะที่การทดลองนี้ใช้ Tryptic soy broth ในการเพาะเลี้ยงเชื้อ ในการใช้ PBS เพาะเลี้ยงเชื้อนั้น พบว่าเชื้อส่วนใหญ่ไม่สามารถเจริญเติบโตต่อไปได้ ดังนั้นการเพาะเลี้ยงเชื้อแบคทีเรียมาตรฐานจึงใช้ Tryptic soy broth เพื่อการเจริญเติบโต

HDL พบอยู่ในทั้งในกระแสเลือด และใน interstitial space ความเข้มข้นของ HDL ในกระแสเลือด พบว่าสูงกว่าใน interstitial fluid ประมาณ 10 เท่า การศึกษานี้ ใช้ความเข้มข้นของ HDL อยู่ในช่วง physiologic range ซึ่งรวมความเข้มข้นที่พบใน interstitial space (~ 100 ไมโครกรัม/มิลลิลิตร) และ ในเลือด(~ 1,670 ไมโครกรัม/มิลลิลิตร) แล้ว อย่างไรก็ตาม การศึกษานี้ไม่ได้ใช้ความเข้มข้นที่เป็น supraphysiologic dose เนื่องจากถูกจำกัดด้วยปริมาณของ HDL ที่แยกออกมาได้

ใน HDL มีโปรตีนหลายชนิดที่พบว่ามีบทบาทเกี่ยวข้องกับการเจริญเติบโตของเชื้อโรค Lipopolysaccharide binding protein (LBP) เป็นโปรตีนตัวหนึ่งของ HDL ที่พบว่าเมื่อฉีดเข้าไปในสัตว์ทดลอง สามารถลดอัตราการตายเมื่อสัตว์ทดลองนั้นกระตุ้นให้เกิดการติดเชื้อด้วยการฉีดแบคทีเรียเข้าไป [28] นอกจากนี้ HDL ยังมีโปรตีนอีกตัวหนึ่งที่เรียกว่า Parotid secretory protein (PSP) ซึ่งพบว่ามีบทบาทยับยั้งการเจริญเติบโตของเชื้อรา Candida albicans ได้ [25]

Apo A-I เป็นโปรตีนอีกตัวหนึ่งของ HDL ซึ่งเป็นโปรตีนหลักที่มีปริมาณมากที่สุดใน HDL และเป็นโครงสร้างของ HDL ด้วย แต่ยังไม่เคยมีการศึกษาผลของ apo A-I ต่อเชื้อแบคทีเรียมาก่อน การศึกษานี้ พบว่า HDL ซึ่งมี apo A-I อยู่ร่วมกับโปรตีนและไขมันอื่นๆอีกหลายชนิด ไม่มีฤทธิ์ยับยั้งการเจริญเติบโตของแบคทีเรีย ในขณะที่ apo A-I อย่างเดียว สามารถยับยั้งการเจริญเติบโตของแบคทีเรียกรัมลบได้ ซึ่งความเข้มข้นของ apo A-I ที่ใช้ในการทดลองนี้ (50 – 200 ไมโครกรัม/มิลลิลิตร) อยู่ในช่วงที่พบได้ในกระแสเลือด สำหรับสาเหตุของความแตกต่างระหว่าง HDL กับ apo A-I ในการยับยั้งการเจริญเติบโตของแบคทีเรียนั้น ยังไม่ทราบแน่ชัด อย่างไรก็ตาม เป็นที่ทราบกันดีว่า apo A-I ที่อยู่ในสภาพอิสระ (free form) มี conformation ของโปรตีนที่ต่างไปจาก apo A-I ที่อยู่รวมกับไขมันอื่นๆ ใน HDL ซึ่ง conformation ที่ต่างกันนี้ อาจมีผลต่อการยับยั้งการเจริญเติบโตของแบคทีเรีย โปรตีน apo A-I มีโครงสร้างที่เป็น amphipathic helices ซึ่งใช้เป็นส่วนที่ associate อยู่กับไขมันต่างๆใน HDL ส่วน

ของ amphipathic helices ของ apo A-I นี้อาจถูกบดบังด้วยไขมันใน HDL ทำให้ไม่สามารถไป associate กับ LPS ในแบคทีเรียได้ แต่เมื่อ apo A-I อยู่ในสภาพอิสระ จึงมี amphipathic helices ที่สามารถไปจับกับ LPS ในแบคทีเรียและส่งผลยับยั้งการเจริญเติบโตได้ มีการศึกษาที่พบว่า apo L-I ซึ่งเป็นโปรตีนอีกตัวหนึ่งของ HDL สามารถยับยั้งการเจริญเติบโตของเชื้อ trypanosome ได้ โดยใช้ส่วนที่เป็น amphipathic helix ในการจับกับโปรตีนของเชื้อ trypanosome [29]

เป็นที่น่าสังเกตว่า มีหลายการศึกษาที่บ่งว่า apo A-I เป็นโปรตีนที่มีส่วนในการลดพิษของ LPS [11-13] โดยพบว่า LPS ที่ incubate กับ apo A-I in vitro สามารถลดการเกิดไข้ที่เกิดจาก LPS ได้เมื่อฉีดเข้าไปในสัตว์ทดลอง [11] สำหรับ in vivo พบว่าหนูทดลองที่มีการแสดงออกของ apo A-I มากกว่าปกติ (transgenic mice overexpressing apo A-I) มีโอกาสรอดชีวิตหลังจากฉีด LPS เข้าไปในร่างกายมากขึ้นกว่าหนูทดลองปกติ [13] การศึกษาที่ผ่านมาทั้งหมด เป็นการศึกษาระหว่าง apo A-I กับ LPS ของแบคทีเรีย แต่การศึกษานี้ *เป็นการศึกษาแรก* ที่พบว่า apo A-I มีผลต่อ*แบคทีเรีย*โดยตรง โดยมีผลยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียกรัมลบ สำหรับผลของ apo A-I ต่อการยับยั้งเชื้อแบคทีเรียกรัมบวกนั้น ได้ผลไม่ชัดเจน ซึ่งอาจเป็นจากลักษณะส่วนประกอบที่ต่างกันระหว่าง LPS ของเชื้อแบคทีเรียกรัมลบกับ LTA ของเชื้อแบคทีเรียกรัมบวก

โดยสรุป การศึกษานี้พบว่า apo A-I สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียกรัมลบในหลอดทดลองได้ ในขณะที่ HDL ไม่มีฤทธิ์ดังกล่าว จึงสมควรที่จะมีการศึกษาเพิ่มเติมของฤทธิ์ของ apo A-I ในสัตว์ทดลอง รวมทั้งค้นคว้าหากลไกการออกฤทธิ์ต่อไป

สรุป

แม้ว่า HDL จะสามารถลดพิษของ LPS และ LTA จากแบคทีเรียได้ ผลการศึกษานี้ พบว่า HDL ทั้งในภาวะปกติและภาวะที่มีการกระตุ้น ไม่มีผลต่อการเจริญเติบโตของแบคทีเรีย อย่างไรก็ตาม apo A-I ซึ่งเป็นโปรตีนหลักของ HDL สามารถยับยั้งการเจริญเติบโตของแบคทีเรียกรัมลบได้ จึงควรที่จะมีการค้นคว้าวิจัยเพิ่มเติมในสัตว์ทดลอง รวมทั้งกลไกการยับยั้งการเจริญเติบโตต่อไป

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1. การเสนอผลงานโปสเตอร์ในการประชุม "นักวิจัยรุ่นใหม่ พบ เมธีวิจัยอาวุโส สกว. 2548" วันที่ 14-16 มกราคม 2548 โรงแรมฟีลิกซ์ ริเวอร์แคว กาญจนบุรี

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3. **Khovidhunkit W**, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of Infection and Inflammation on Lipid and Lipoprotein Metabolism: Mechanisms and Consequences to the Host. *Journal of Lipid Research*. 2004;45:1169-96. Impact factor 4.159

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thematic review

Thematic review series: The Pathogenesis of Atherosclerosis

Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host[1]

Weerapan Khovidhunkit,* Min-Sun Kim,† Riaz A. Memon,[1,†] Judy K. Shigenaga,† Arthur H. Moser,† Kenneth R. Feingold,[2,†] and Carl Grunfeld[2,†]

Division of Endocrinology and Metabolism,* Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand; and Department of Medicine,† University of California, San Francisco, and Metabolism Section, Medical Service, Department of Veterans Affairs Medical Center, San Francisco, CA

Abstract Infection and inflammation induce the acute-phase response (APR), leading to multiple alterations in lipid and lipoprotein metabolism. Plasma triglyceride levels increase from increased VLDL secretion as a result of adipose tissue lipolysis, increased de novo hepatic fatty acid synthesis, and suppression of fatty acid oxidation. With more severe infection, VLDL clearance decreases secondary to decreased lipoprotein lipase and apolipoprotein E in VLDL. In rodents, hypercholesterolemia occurs attributable to increased hepatic cholesterol synthesis and decreased LDL clearance, conversion of cholesterol to bile acids, and secretion of cholesterol into the bile. Marked alterations in proteins important in HDL metabolism lead to decreased reverse cholesterol transport and increased cholesterol delivery to immune cells. Oxidation of LDL and VLDL increases, whereas HDL becomes a proinflammatory molecule. Lipoproteins become enriched in ceramide, glucosylceramide, and sphingomyelin, enhancing uptake by macrophages. Thus, many of the changes in lipoproteins are proatherogenic. The molecular mechanisms underlying the decrease in many of the proteins during the APR involve coordinated decreases in several nuclear hormone receptors, including peroxisome proliferator-activated receptor, liver X receptor, farnesoid X receptor, and retinoid X receptor. APR-induced alterations initially protect the host from the harmful effects of bacteria, viruses, and parasites. However, if prolonged, these changes in the structure and function of lipoproteins will contribute to atherogenesis. Khovidhunkit, W., M-S. Kim, R. A. Memon, J. K. Shigenaga, A. H. Moser, K. R. Feingold, and C. Grunfeld. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J. Lipid Res.* 2004. 45: 1169–1196.

Supplementary key words acute-phase response • endotoxin • lipopolysaccharide • cytokine • atherosclerosis

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The acute-phase response (APR), an early, highly complex reaction of the host, is induced by injurious stimuli including infection and inflammation, trauma, burns, ischemic necrosis, and malignant growth (1). The APR is

Abbreviations: ABC, ATP binding cassette; ACC, acetyl CoA carboxylase; ACS, acyl-CoA synthetase; AIDS, acquired immune deficiency syndrome; AP2, adipocyte P2; apoE, apolipoprotein E; APR, acute-phase response; BSEP, bile salt export pump; CAD, coronary artery disease; CAR, constitutive androstane receptor; CETP, cholesteryl ester transfer protein; CNTF, ciliary neurotropic factor; CPT, carnitine palmitoyl transferase; CRP, C-reactive protein; CYP7A1, cholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; CYP8B1, sterol 12α hydroxylase; CYP27A1, sterol 27-hydroxylase; DR, direct repeat; ERK, extracellular signal-related kinase; EL, endothelial lipase; FABP, fatty acid binding protein; FAS, fatty acid synthase; FAT, fatty acid translocase; FATP, fatty acid transport protein; FXR, farnesoid X receptor; GlcCer, glucosylceramide; GSL, glycosphingolipid; HIV, human immunodeficiency virus; HNF, hepatocyte nuclear factor; HSL, hormone-sensitive lipase; IL, interleukin; KB, ketone body; KGF, keratinocyte growth factor; LBP, lipopolysaccharide binding protein; LIF, leukemia inhibitory factor; Lp[a], lipoprotein [a]; LPC, lysophosphatidylcholine; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LRH-1, liver receptor homolog-1; LTA, lipoteichoic acid; LXR, liver X receptor; MDR3, multidrug resistance-3; MEK, mitogen-activated protein kinase kinase; MRP2, multidrug resistance-associated protein-2; NF-κB, nuclear factor κB; NF-IL-6, nuclear factor interleukin-6; NGF, nerve growth factor; OATP, organic anion transporting protein; oxPAPC, oxidized 1 palmitoyl 2 arachidonoyl sn-glycero 3 phosphorylcholine; PAF, platelet-activating factor; PAF-AH, platelet activating factor acetylhydrolase; PKA, protein kinase A; PLTP, phospholipid transfer protein; PON1, paraoxonase 1; PPAR, peroxisome proliferator-activated receptor; PTHrP, parathyroid hormone-related protein; PXR, pregnane X receptor; RCT, reverse cholesterol transport; RXR, retinoid X receptor; SAA, serum amyloid A; SHP, small heterodimer partner; sPLA$_2$, secretory phospholipase A$_2$; SPT, serine palmitoyltransferase; SR-BI, scavenger receptor class B type I; TG, triglyceride; TLF, trypanosome lytic factor; TNF, tumor necrosis factor; TR, thyroid hormone receptor.

This paper is dedicated to the memory of Dr. Riaz A. Memon, deceased.

[1] To whom correspondence should be addressed.
e-mail: grunfeld@itsa.ucsf.edu (C.G.)
kfeingold@itsa.ucsf.edu (K.R.F.)

accompanied by specific changes in the concentration of plasma proteins. Proteins that increase by at least 25% during the APR are positive acute-phase proteins [e.g., C-reactive protein (CRP), serum amyloid A (SAA), and fibrinogen], whereas proteins that decrease are negative acute-phase proteins (e.g., albumin, transferrin, and α-fetoprotein) (1). Changes in acute-phase protein concentrations are largely attributable to alterations in their rate of synthesis in the liver, although similar changes occur in extrahepatic tissues. Microarrays of mouse liver after endotoxin treatment demonstrate that ~7% of the genes respond to endotoxin challenge (2). These changes in acute-phase proteins are often species specific with regard to the magnitude and direction of change.

The APR induced during infection/inflammation protects the host from further injury (1). Changes in acute-phase proteins neutralize invading microorganisms, minimize the extent of tissue damage, participate in the local immune response and tissue regeneration, and replenish proteins used in the inflammatory process. These changes, if present for a prolonged period of time, can lead to detrimental consequences to the host, such as the development of systemic amyloidosis after chronic infection or inflammation.

Changes in acute-phase protein synthesis are mediated by cytokines produced in response to a variety of stimuli in multiple cell types, including macrophages, monocytes, T-lymphocytes, and endothelial cells (1). Key cytokines responsible for the coordination of both immune and inflammatory responses include tumor necrosis factors (TNF-α and TNF-β), interleukins (ILs), and interferons (IFN-α, -β, and -γ) (1). Redundancy classically occurs in essential parts of the host response, as several structurally different cytokines may exert similar biological effects even though they bind to different receptors. Combinations of certain cytokines produce additive or synergistic effects, whereas other cytokines may have inhibitory effects, indicating the complex nature of the host response (3–5).

Infection and inflammation are accompanied by similar cytokine-induced alterations in lipid and lipoprotein metabolism. Of note, inflammatory cytokines are increased and play a pathogenic role in a variety of very common disorders, such as diabetes, obesity, metabolic syndrome, hypertension, chronic heart failure, chronic renal failure, and atherosclerosis (6–12). Many of these disorders display abnormalities in lipid metabolism that are similar to those that occur during infection and inflammation.

This review summarizes the changes in lipid and lipoprotein during infection/inflammation and their molecular mechanisms. Most mechanistic studies were carried out in animal models of infection using endotoxin [lipopolysaccharide (LPS)], a well-characterized inducer of cytokines and the APR, or the proinflammatory cytokines (TNF and IL-1), which mediate the APR. We describe the role of transcription factors in regulating lipid metabolism during infection/inflammation. Finally, we discuss both the beneficial effects and deleterious consequences to the host of APR-induced changes in lipid and lipoprotein metabolism.

CHANGES IN LIPID AND LIPOPROTEIN METABOLISM DURING INFECTION AND INFLAMMATION

An early and consistent metabolic alteration during infection/inflammation is increased serum triglyceride (TG) levels, characterized by an increase in VLDL levels (13). Multiple mechanisms produce hypertriglyceridemia during the APR; several cytokines are capable of producing these changes. Whether an increase in glucocorticoid levels during infection plays a role in lipid metabolism is unclear.

The effects of infection and inflammation on TG metabolism are similar in all species, whereas changes in cholesterol metabolism differ between rodents and primates. In rodents, there is an increase in serum total cholesterol levels and hepatic cholesterol synthesis, whereas nonhuman primates and humans have either no change or a decrease in serum cholesterol and LDL levels (13). The mechanisms underlying this species difference is not known. HDL levels are decreased in both rodents and primates during the APR, and there are marked changes in proteins associated with HDL metabolism (14). Finally, infection produces alterations in the composition and function of lipoproteins, including changes in sphingolipid concentrations, decreased reverse cholesterol transport (RCT), and increased oxidation of lipids.

TG metabolism

Patients with gram-negative or gram-positive bacterial infections and viral infections have increased serum TG levels (15–17). In animals, administration of LPS, a major component of the cell wall of gram-negative bacteria, or lipoteichoic acid (LTA), a component of the cell wall of gram-positive bacteria, produces hypertriglyceridemia (18–28) (**Table 1**). Multiple cytokines increase serum TG levels in rodents and in humans (29–40). The hypertriglyceridemic effect of LPS and cytokines is rapid, occurring within 2 h after administration, and is sustained for at least 24 h (26, 29). The doses of LPS or cytokines that produce hypertriglyceridemia in rodents are similar to those that produce fever, anorexia, and changes in acute-phase protein synthesis, suggesting that hypertriglyceridemia is a very sensitive, physiological part of the host response to infection rather than a manifestation of toxicity (26).

The increased VLDL is secondary to either increased VLDL production or decreased VLDL clearance, depending upon the dose of LPS (26). At low doses, VLDL production increases as a result of increased hepatic FA synthesis, activation of adipose tissue lipolysis, and suppression of FA oxidation and ketogenesis. All of these mechanisms provide more FA substrate in the liver for esterification into TG and secretion as VLDL. At higher doses of LPS, VLDL clearance is decreased as a result of decreases in the activity of lipoprotein lipase (LPL), the enzyme responsible for the catabolism of TG-rich lipoproteins, and decrease in levels of apolipoprotein E (apoE).

Serum TG levels are increased by multiple cytokines, including TNF, IL-1, IL-2, IL-6, leukemia inhibitory factor

TABLE 1. Effects of LPS, LTA, and cytokines on TG metabolism in intact animals

Variable	LPS	LTA	TNF	IL-1	IL-6	IFN-α	IFN-γ
Serum TG	↑	↑	↑	↑	↑	↔	↔
Hepatic FA synthesis	↑	↑	↑	↑	↑	↑	↔
TG secretion	↑	↑	↑	↑	↑	ND	ND
Lipolysis	↑	↑	↑	↔	↑	↑	↑
FA oxidation	↓	ND	↓	↓	ND	ND	ND
Serum ketone body	↓	ND	↓	↓	↔	↑[a], ↔[b]	↑
TG clearance	↔[a], ↓[b]	↔	↔	↔	↔	ND	ND
LPL activity	↓	↔	↓, ↔[c]	↓	↓	↓	↓

IL, interleukin; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LTA, lipoteichoic acid; ND, not determined; TG, triglyceride; TNF, tumor necrosis factor.

[a] Low doses.

[b] High doses.

[c] Some but not most tissues.

(LIF), ciliary neurotropic factor (CNTF), nerve growth factor (NGF), keratinocyte growth factor (KGF), platelet-activating factor (PAF), and parathyroid hormone-related protein (PTHrP) (30, 32–39, 41–47) (Table 1), suggesting redundancy. IL-4, an anti-inflammatory cytokine, opposes the action of some, but not all, of these cytokines (48). The effects of cytokines on TG metabolism are likely direct and not mediated by hormones such as insulin, cortisol, or catecholamines, as TNF increases serum TG levels in insulinopenic diabetic animals and adrenalectomized rats (49, 50). Moreover, TNF also increases serum TG levels under various dietary conditions from high sucrose, which stimulates endogenous FA synthesis, to high fat, which suppresses endogenous FA synthesis (51, 52).

Increased VLDL production. INCREASED DE NOVO FA AND TG SYNTHESIS. LPS and several cytokines, including TNF-α, TNF-β (lymphotoxin), IL-1, IL-6, IFN-α, LIF, CNTF, NGF, PAF, and PTHrP, rapidly induce de novo FA synthesis and hepatic TG synthesis in rodents (26, 29, 31, 32, 34, 43, 44, 47, 50, 53) (Table 1). Hepatic secretion of apoB also increases (54), resulting in an increased number of VLDL particles secreted. In contrast, other cytokines, such as IL-2, IL-4, and IFN-γ, do not stimulate hepatic FA synthesis (31, 48).

TNF rapidly increases hepatic FA synthesis within 1 h after administration, which is sustained for at least 17 h (29). The time course for stimulation of hepatic FA synthesis and VLDL secretion is consistent with the time course for TNF-induced hypertriglyceridemia (29, 55). However, TNF does not acutely increase the total activity of the rate-limiting enzymes of FA synthesis [i.e., acetyl CoA carboxylase (ACC) and FA synthase (FAS)] or alter the phosphorylation state of ACC, a mechanism that regulates ACC activity (56). Instead, TNF acutely increases intracellular concentrations of citrate, an allosteric activator of ACC (56) (Fig. 1). IL-1 and IL-6 increase hepatic FA synthesis by increasing hepatic citrate levels, whereas IFN-α, which also increases hepatic FA synthesis, has no effect on citrate levels, suggesting a different mechanism (53). The stimulatory effects of TNF or IL-1 and IFN-α on hepatic FA synthesis are additive or synergistic, whereas there is no such synergy between TNF and IL-1 or TNF and IL-6 (53). Finally, IL-4, an anti-inflammatory cytokine, inhibits the stimulatory effects of TNF, IL-1, and IL-6 on hepatic FA synthesis by blocking the increase in hepatic citrate levels (48). In contrast, IL-4 does not block the stimulatory effect of IFN-α on FA synthesis in liver (48). Thus, analogous to cytokine regulation of the immune response, there are complex interactions among the metabolic effects of cytokines that may be additive, synergistic, or antagonistic.

The late effects of TNF on hepatic FA synthesis are accompanied by modest increases in hepatic ACC and FAS activities (56). Late increases in ACC activity in rat liver occur in a sepsis model induced by cecal ligation and puncture (57). Whether gene expression of ACC and/or FAS increases in the liver is currently not known.

INCREASED ADIPOSE TISSUE LIPOLYSIS. Adipose tissue lipolysis also provides FAs for increased hepatic TG synthesis during infection. The mobilized FAs are delivered to the liver and, instead of being oxidized, become reesterified into TGs and secreted into the circulation as VLDL.

LPS, LTA, and several cytokines induce adipose tissue lipolysis in both intact animals and 3T3-L1 adipocytes (26,

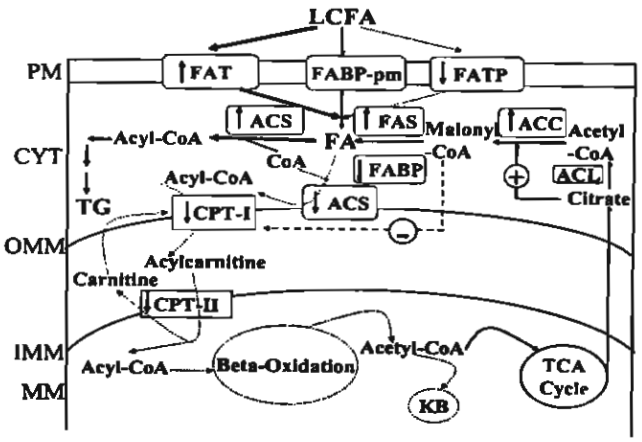

Fig. 1. Changes in hepatic FA metabolism during the acute-phase response (APR). Lipopolysaccharide (LPS) and cytokines increase CD36/fatty acid translocase (FAT) while decreasing fatty acid-transport protein (FATP) in the liver. CD36/FAT may transport long chain FA (LCFA) to cytosol for reesterification, which is enhanced during infection and inflammation, whereas FATP may transport FA toward mitochondria for oxidation, which is suppressed during infection. Cytokines, such as tumor necrosis factor and interleukin-1, increase hepatic FA synthesis by increasing hepatic citrate levels. Modest increases in acetyl CoA carboxylase (ACC) and FA synthase (FAS) are also observed. The expression of carnitine palmitoyl transferase-I (CPT-I) and CPT-II is decreased during sepsis. In addition, LPS and cytokines increase the levels of hepatic malonyl CoA, which further inhibits CPT-I, the rate-limiting enzyme in FA oxidation, resulting in decreased FA oxidation and suppressed ketone body (KB) production in the liver. ACS, acyl-CoA synthetase; CYT, cytosol; IMM, inner mitochondrial membrane; MM, mitochondrial matrix; OMM, outer mitochondrial membrane; PM, plasma membrane; TG, triglyceride.

28, 34, 37, 43, 45, 52, 58–63). The effects of different cytokines are specific and dependent upon the nutritional status of the host (Table 1). TNF acutely induces lipolysis in chow-fed but not in sucrose-fed animals (52). IL-1 does not stimulate lipolysis; its effect on serum TG levels is attributable to enhanced hepatic FA synthesis and TG secretion (32). IL-6, LIF, and CNTF, which act through the same receptor transducer (gp130), stimulate both hepatic FA synthesis and adipose tissue lipolysis (34, 43). On the other hand, KGF stimulates lipolysis but has no effect on hepatic FA synthesis (45). Finally, both IFN-α and IFN-γ stimulate lipolysis, but those peripherally derived FAs do not contribute to increased TG synthesis in the liver because they are oxidized, producing ketone bodies (KBs) (63).

Lipolysis in adipose tissue is primarily driven by hormone-sensitive lipase (HSL), which is regulated either by alteration in its phosphorylation state or by induction of gene expression. Several cytokines that induce lipolysis, including TNF, IFN-α, and IFN-γ, produce a marked decrease in HSL mRNA (64), indicating that gene regulation of HSL does not play a role in cytokine-induced lipolysis. Rather, lipolysis is likely attributable to phosphorylation of HSL or its associated proteins. TNF-induced lipolysis in cultured human adipocytes is associated with the activation of mitogen-activated protein kinase kinase (MEK)-extracellular signal-related kinase (ERK) (65), leading to decreases in cyclic nucleotide phosphodiesterase 3B, an enzyme that hydrolyzes cAMP. Increased intracellular cAMP consequently activates cAMP-dependent protein kinase A (PKA), which phosphorylates perilipins, phosphoproteins located at the surface of lipid droplets in adipocytes. Phosphorylation of perilipin A or B modifies lipid surfaces, allowing access of lipases to the lipid droplets, promoting lipolysis. Activation of the MEK-ERK pathway and PKA has also been shown to phosphorylate HSL and increase its lipolytic activity (65, 66).

LPS and cytokines may also induce lipolysis by decreasing the expression of acyl-CoA synthetase (ACS) in adipose tissue (64). ACS catalyzes the activation of long-chain FAs to acyl-CoA esters that are subsequently metabolized in anabolic or catabolic pathways depending on the type of tissue, the nutritional status, and the hormonal milieu of the host. Although FA transport across biological membranes is a bidirectional process, activation of FAs to acyl-CoA esters prevents the efflux of FAs from cells and hence renders FA transport unidirectional. In adipose tissue, ACS is primarily associated with microsomes to support the synthesis of TG for storage of energy. During the APR, there is a coordinated decrease in the mRNA expression of fatty acid transport proteins (FATPs) and ACS mRNA and activity in adipose tissue (67, 68) that likely prevents the activation and storage of FAs and may promote the mobilization of FAs.

DECREASED HEPATIC FA OXIDATION AND KETOGENESIS. Bacterial infections are accompanied by the suppression of hepatic FA oxidation (69, 70). Increased FA substrate provided by increased hepatic FA synthesis and adipose tissue lipolysis is then directed away from oxidation and channeled toward reesterification. This concept is supported by the demonstration that LPS, TNF, and IL-1 decrease mitochondrial but increase microsomal ACS activity in the liver (68). Decreased mitochondrial ACS prevents the activation of FA for entry into mitochondria for oxidation, whereas increased microsomal ACS enhances the reesterification of FAs for TG synthesis.

LPS and cytokines differentially regulate the hepatic mRNA expression of membrane-associated FATPs involved in the uptake of peripherally derived FAs. LPS and cytokines increase the expression of CD36/fatty acid translocase (FAT) while decreasing the mRNA levels of FATP in the liver, suggesting that these proteins may be involved in directing FAs to different intracellular locations (67) (Fig. 1). We propose that CD36/FAT transports FAs to cytosol for reesterification, which is enhanced during the APR, whereas FATP transports FAs toward mitochondria for oxidation, which is suppressed during the APR. LPS also decreases the mRNA and protein levels of cytosolic fatty acid binding protein (FABP) in liver, heart, and muscle (71). Because FABPs are thought to facilitate the transport of FAs to the site of utilization in the cell, the decrease in FABP may also contribute to decreased FA oxidation during infection. The fact that TNF does not acutely increase the activities of regulatory enzymes of TG synthesis, such as glycerol phosphate acyltransferase and diacylglycerol acyltransferase (52), also suggests that the acute increase in TG synthesis is driven by increased FA substrate.

Mitochondrial ACS converts FA into fatty acyl-CoA, which is subsequently metabolized by mitochondrial carnitine palmitoyl transferase-I (CPT-I) into acylcarnitine. CPT-II subsequently metabolizes acylcarnitine into acyl-CoA, which allows FA entrance into the mitochondria, where it undergoes β-oxidation. Hepatic expression of both CPT-I, the rate-limiting enzyme for mitochondrial FA oxidation, and CPT-II is decreased during sepsis (72, 73) (Fig. 1). LPS, IL-1, and TNF increase levels of hepatic malonyl-CoA, an allosteric inhibitor of CPT-I, which inhibits the remaining CPT-I, decreasing FA oxidation (74) (Table 1). IFN-α at high doses increases hepatic malonyl-CoA levels (63), whereas IFN-γ does not affect hepatic malonyl-CoA levels (63).

Given the decrease in FA oxidation, infection is associated with the suppression of hepatic KB production (69, 70). Serum KB levels are regulated by their rates of synthesis in the liver and utilization in peripheral tissues. Infection decreases KB production through the inhibition of FA oxidation but also likely by increased peripheral KB utilization.

Various cytokines have different effects on KB metabolism (Table 1). Both TNF and IL-1 acutely decrease serum KB levels in mice (30, 74). In the fed state, IL-1 increases hepatic malonyl-CoA levels, inhibiting CPT-I and preventing KB production. During fasting, IL-1 inhibits lipolysis, reducing FA substrate to the liver for KB synthesis (74). Although TNF increases hepatic malonyl-CoA levels, it stimulates peripheral lipolysis, increasing the flux of FA substrate to the liver, with no net effect on hepatic KB levels (74), suggesting that TNF decreases serum KB through

changes in KB catabolism. IL-6 has no effect on serum KB levels (34). IFN-α has biphasic effects: low doses of IFN-α increase serum KB levels by mobilization of FA substrate, whereas higher doses have no effect (63). IFN-γ stimulates adipose tissue lipolysis, increasing serum and hepatic KB levels (63).

FA uptake and oxidation decrease in heart and skeletal muscle during the APR, shifting their metabolism from FA as the preferred fuel substrate to glucose, whose uptake and utilization are increased (75–77). This makes more FA available to liver and other tissues, such as those of the immune system. IL-1, but not TNF, decreases LPL activity in the heart (78–80). LPS, TNF, and IL-1 decrease the mRNA expression of FA transport and binding proteins and ACS in heart and muscle (67, 71). It is likely that this coordinated decrease in FA transport and binding proteins and ACS is the mechanism for the decreased uptake and utilization of FA in heart and muscle during infection/inflammation.

Decreased VLDL clearance. Infection may also increase serum TG levels by decreasing VLDL clearance. Early in vitro studies showed that TNF decreases LPL expression in cultured adipocytes (81, 82). In vivo, however, there is little evidence that hypertriglyceridemia is attributable to decreased LPL activity. First, although TNF reduces LPL activity in epididymal fat pads in rodents (80, 83), this decrease requires many hours, whereas the TNF-induced increase in serum TG levels occurs very rapidly (29). Second, TNF administration does not decrease LPL activity in other adipose tissue sites or in muscle (41, 83). Third, TNF-neutralizing antibodies block the LPS-induced increase in serum TG levels in mice but they do not block LPS-induced inhibition of LPL in mouse adipose tissue, again dissociating the LPS-induced increase in serum TGs from changes in LPL activity (33). Finally, TNF does not decrease the clearance of chylomicrons or VLDL from the circulation, the mechanism by which changes in LPL might influence TG levels (41, 49, 84).

Like TNF, IL-1, IL-6, and LIF also require several hours to decrease LPL activity in vivo in mouse adipose tissue (80). IFN-α and IFN-γ increase serum TG levels in humans (38, 39) but do not increase TG levels in rodents, despite decreasing LPL activity in cultured murine 3T3-L1 fat cells (64, 82), again showing discordance between LPL activity and TG levels.

There may be a role for the decreased clearance of TG with high doses of LPS. Low doses of LPS enhance hepatic VLDL secretion and increase serum TG levels without affecting TG clearance in rats. In contrast, high doses of LPS inhibit the clearance of TG-rich lipoproteins (26). Moreover, high doses of LPS decrease postheparin plasma LPL activity and LPL activity in adipose tissue and muscle (80).

LPS and cytokines also decrease apoE mRNA in many tissues, including the liver, and VLDL has lower amounts of apoE during infection (54, 85, 86). Because apoE is required for the clearance of TG-rich lipoproteins, decreased apoE could contribute to the delayed clearance observed in rats with infection (87).

Cholesterol metabolism

There are marked alterations in the metabolism of cholesterol, LDL, HDL, and RCT during infection. LPS and cytokines decrease total serum cholesterol levels in primates, whereas in rodents they increase cholesterol levels by stimulating de novo cholesterol synthesis, decreasing lipoprotein clearance, and decreasing the conversion of cholesterol into bile acids. Such species-specific responses in the APR are common, but the underlying mechanisms responsible for these differences are not yet understood. There are baseline differences in serum cholesterol levels among species, with rodents having low LDL levels and primates having relatively high LDL levels. Baseline levels are often related to the direction of changes in the APR. There are classic positive acute-phase proteins that are expressed at baseline in some species, and they do not increase during the APR in those species.

Hepatic cholesterol synthesis. In rodents, LPS stimulates hepatic cholesterol synthesis (27) (**Table 2**). In contrast to the acute effect of LPS on de novo FA synthesis, the effect of LPS on hepatic cholesterol synthesis is delayed, occurring 16 h after administration (27). LPS stimulates hepatic cholesterol synthesis by increasing the transcription rate, mRNA expression, protein mass, and activity of HMG-CoA reductase, the rate-limiting enzyme in the biosynthetic pathway of cholesterol liver (27, 88). The effect of LPS on HMG-CoA reductase is specific, as the mRNA expression of several other enzymes in the cholesterol synthetic pathway, including HMG-CoA synthase and farnesyl pyrophosphate synthase, which are usually coordinately regulated with HMG-CoA reductase under nutritional or pharmacological manipulations, is not altered by LPS treatment (2, 88) (**Fig. 2**). Moreover, LPS still upregulates HMG-CoA reductase mRNA expression when its basal expression is increased by treatment with bile acid binding resins or decreased by feeding a high-cholesterol diet (88). Thus, the stimulatory effect of LPS on HMG-CoA reductase is independent of dietary regulation and persists over a wide range of basal expression.

Despite a marked increase in HMG-CoA reductase activity, LPS only produces a modest increase in hepatic cholesterol synthesis and serum cholesterol levels (27). The reason is that LPS produces a decrease in the mRNA expression and activity of squalene synthase (89), the first

TABLE 2 Effects of LPS, LTA, and cytokines on cholesterol metabolism in intact animals

Variable	LPS	LTA	TNF	IL-1	IL-6	IFN-α	IFN-γ
Serum cholesterol	↑, ↓[a]	↑	↑, ↓[a]	↑, ↔[a]	↑	↔	↔
Hepatic cholesterol synthesis	↑	ND	↑	↑	ND	↔	↑
HMG-CoA reductase activity	↑	ND	↑	↑	ND	ND	↔
LDL receptor protein	↓, ↔[b]	ND	↔[b]	↔[b]	ND	ND	ND
Bile acid synthesis	↓	ND	↓	↓	ND	ND	ND

Data are for rats and mice unless otherwise noted.
[a] Primates.
[b] Hamsters.

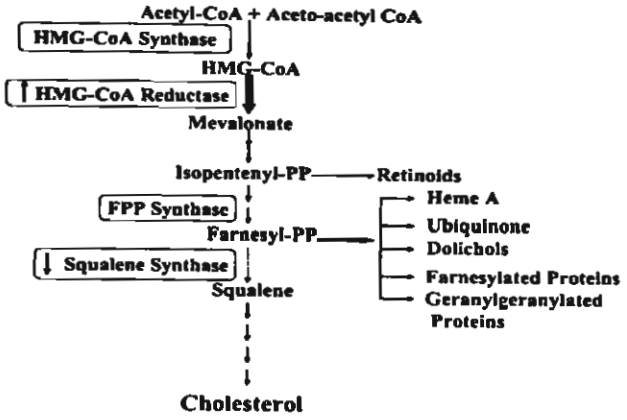

Fig. 2. Changes in cholesterol metabolism during the APR. Infection and inflammation are associated with an increase in HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis in the liver. However, there is a decrease in the expression of enzymes downstream of the mevalonate pathway, including squalene synthase. As a result, there is only a modest increase in hepatic cholesterol synthesis, and other mevalonate metabolites are redirected into nonsterol pathways, such as dolichols. FPP, farnesyl pyrophosphate.

committed enzyme in cholesterol synthesis located at a branch point in the mevalonate pathway (Fig. 2), and other enzymes downstream of mevalonate pathway (2). Regulation of squalene synthase plays an important role in regulating the flux of metabolic intermediates to the sterol or nonsterol pathways, which include the synthesis of retinoids, dolichols, ubiquinone, and prenylated proteins. It is likely that the LPS-induced increase in HMG-CoA reductase coupled with a decrease in squalene synthase maintains adequate cholesterol synthesis while redirecting mevalonate metabolites into nonsterol pathways (Fig. 2). Indeed, the synthesis of dolichol phosphate is increased in the liver during inflammation (90, 91). Dolichol is required for the glycosylation of proteins, and the synthesis of several glycosylated plasma proteins is markedly increased in the liver during the APR (90, 91).

Like LPS, several cytokines, including TNF, IL-1, IL-6, KGF, and NGF, produce a delayed increase in serum cholesterol levels in rodents (29, 32, 34, 44, 45) (Table 2). TNF-α, TNF-β, IL-1, and IFN-γ stimulate hepatic cholesterol synthesis in mice, whereas IFN-α and IL-2 have no such effect (31). Like LPS, both TNF and IL-1 stimulate de novo hepatic cholesterol by increasing the activity and mRNA expression of HMG-CoA reductase (88, 92). TNF and IL-1 decrease squalene synthase activity and mRNA expression (89); they may also divert the flux of mevalonate metabolites into nonsterol pathways during the APR.

In primates, including humans, infection/inflammation decreases serum cholesterol as a result of decreases in both LDL and HDL cholesterol (16, 17, 24, 25). LPS, TNF, IL-2, IFN-β, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor decrease serum cholesterol, whereas IL-1 has no effect (24, 25, 36, 42, 93–97). The decrease in cholesterol is accompanied by a reduction in serum apoB levels.

The mechanism by which infection/inflammation decreases cholesterol levels has not been thoroughly studied in intact primates. Most of the mechanistic studies were performed in vitro using human hepatoma HepG2 cells. IL-1 inhibits cholesterol synthesis and decreases cholesterol and apoB secretion, whereas IL-6 increases cholesterol synthesis but decreases cholesterol secretion (98). IFN-β also decreases apoB synthesis (99).

LDL clearance. In rats, LPS significantly inhibits the clearance of LDL from the circulation (100). LPS decreases the expression of hepatic LDL receptor protein (Table 2), but the decrease in protein levels could not be explained by changes in mRNA levels, suggesting that posttranscriptional regulation occurs during the APR (101). In a rat model of gram-negative sepsis, the rate of apoB degradation is decreased (87). In hamsters, however, LPS, IL-1, and TNF either have no effect or produce a slight increase in hepatic LDL receptor mRNA and protein levels (27). In human HepG2 cells, IL-1 and TNF increase LDL receptor activity (102, 103). The differences may explain the species-specific response in cholesterol metabolism commonly seen during the APR.

Decreased hepatic cholesterol catabolism and excretion. Equipped with a number of enzymes and transporters, hepatocytes secrete bile salts, phospholipids, cholesterol, organic anions, and cations into the bile. Cholesterol returned to the liver is primarily metabolized into bile acids, representing the major pathway for the elimination of cholesterol from the body. There are two distinct pathways of bile acid synthesis in mammalian liver (104, 105). The classic or neutral pathway is initiated by microsomal cholesterol 7α-hydroxylase (CYP7A1) that converts cholesterol into 7α-hydroxycholesterol, which is subsequently converted into primary bile acids. The alternative or acidic pathway is initiated by mitochondrial sterol 27-hydroxylase (CYP27A1) that converts cholesterol into 27-hydroxycholesterol, which is then converted into 7α,27-dihydroxycholesterol by oxysterol 7α-hydroxylase (CYP7B1) and subsequently metabolized into primary bile acids. The alternative pathway may contribute as much as 50% to total bile acid synthesis (104, 105). Primary bile acids synthesized in hepatocytes are conjugated with taurine and glycine. At physiological pH, these conjugates exist in the anionic salt form; therefore, they are called bile salts. Secretion of bile salts mediates the solubilization of lipids from the canalicular membrane, resulting in the secretion of biliary phospholipids and cholesterol.

As polarized cells, hepatocytes contain multiple transporters at the basolateral (sinusoidal) and apical (canalicular) surfaces (106). Basolateral bile salt uptake from the portal circulation is primarily mediated by sodium taurocholate-cotransporting protein. Several organic anion-transporting proteins (OATPs), including OATP1, OATP2, and OATP4, are also involved in sodium-independent bile salt uptake. At the canalicular surface, bile salt secretion into the bile duct is mediated by members of the ATP binding cassette (ABC) superfamily. An ABC transporter hydrolyzes intracellular ATP to transport biliary components against the concentration gradient into the

bile. The canalicular bile salt export pump (BSEP or ABCB11) secretes monovalent bile salts, whereas multidrug resistance-associated protein-2 (MRP2 or ABCC2) secretes divalent bile salts. Once secreted into the bile, bile salts stimulate the secretion of phospholipids and cholesterol from the canalicular membrane, forming micelles. Multidrug resistance-3 (MDR3 or ABCB4 in humans or MDR2 in rodents) is a phospholipid transporter. Secretion of intact cholesterol into bile is mediated by a heterodimer of two ABC transporters, ABCG5 and ABCG8 (107, 108). These transporters are transcriptionally regulated by a variety of nuclear hormone receptors (106).

LPS and cytokines decrease the catabolism and excretion of cholesterol. In the liver, LPS markedly decreases the mRNA expression and activity of CYP7A1, the rate-limiting enzyme in the classic pathway of bile acid synthesis (109) (**Fig. 3**). This effect is very rapid, occurring within 90 min of LPS administration, and is sustained for at least 16 h (109). LPS also decreases the mRNA expression and activity of CYP27A1, the rate-limiting enzyme in the alternative pathway of bile acid synthesis, and mRNA levels of CYP7B1 in the liver (110) (Fig. 3). The decreases in CYP27A1 and CYP7B1 occur 8–16 h after LPS administration and persist for at least 24 h, suggesting that both the classic and alternative pathways of bile acid synthesis are sequentially downregulated during infection and inflammation. Like LPS, both TNF and IL-1 also decrease hepatic CYP27A1 and CYP7B1 mRNA expression (110).

Infection is associated with intrahepatic cholestasis that may be attributable to effects on biliary transport. LPS administration in rodents reduces bile salt uptake, bile salt secretion, and bile flow, which are mediated by decreases in the expression of several transporters involved in the hepatocellular uptake, including NCTP, OATP1, and OATP2 (111–114), and canalicular excretion of bile salts, including BSEP and MRP2 (114, 115). LPS and cytokines also decrease the expression of MDR2 in rats, which mediates phospholipid secretion into bile (114, 116). Moreover, LPS coordinately decreases hepatocyte mRNA levels for ABCG5 and ABCG8, which mediate cholesterol excretion into the bile (117). Thus, biliary secretion of bile salts, phospholipids, and cholesterol are all impaired during infection. Figure 3 summarizes the effect of APR on bile acid metabolism.

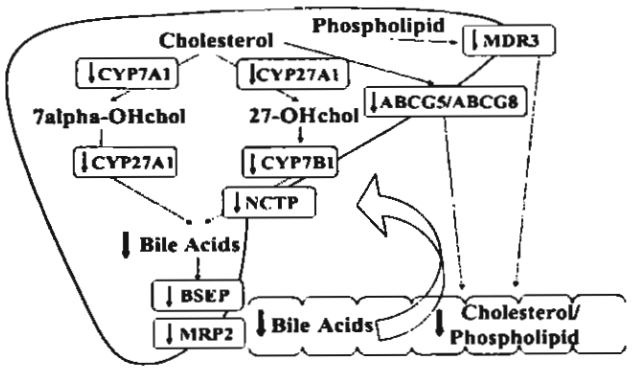

Fig. 3. Changes in bile acid metabolism during the APR. LPS and cytokines decrease the catabolism and excretion of cholesterol in the liver by decreasing the expression and activities of enzymes in both the classic pathway and the neutral pathway, including cholesterol 7α-hydroxylase (CYP7A1), sterol 27-hydroxylase (CYP27A1), oxysterol 7α-hydroxylase (CYP7B1), and sterol 12α-hydroxylase. LPS also decreases the expression of several protein transporters involved in the canalicular excretion of bile salts, such as bile salt export pump (BSEP) and multidrug resistance-associated protein-2 (MRP2), and those in the hepatocellular uptake of bile salts, including sodium taurocholate-cotransporting protein and organic anion-transporting proteins. Furthermore, LPS decreases the excretion of cholesterol and phospholipids into the bile by downregulating ABCG5/ABCG8 and multidrug resistance-3 (MDR3), respectively.

The coordinated downregulation of both pathways of bile acid synthesis during the APR is in contrast to most other situations, including studies in knockout animals, in which during the suppression or absence of one pathway of bile acid synthesis the enzymes of the other pathway are upregulated to compensate for the deficiency. The decreases in the regulatory enzymes of both the classic and alternative pathways of bile acid synthesis as well as the decrease in ABCG5 and ABCG8 induced by LPS and cytokines suggest that during infection the body's need to conserve cholesterol is so essential that all of these pathways are downregulated to limit the elimination of cholesterol from the body. A decrease in cholesterol catabolism would make cholesterol more available for hepatic lipoprotein production.

Lipoprotein [a]. Lipoprotein [a] (Lp[a]) is a distinct lipoprotein consisting of an LDL particle attached to apo[a] that is present in primates but not in rodents and most other species (118). Lp[a] is cholesterol-rich; increased serum levels have been associated with a higher risk for atherosclerosis. The physiological role of Lp[a] is not known, but it is thought to be involved in wound healing. The structure of apo[a] resembles plasminogen, and apo[a] has been found in the lesions during early stages of wound healing. Alternatively, Lp[a] may act as a scavenger of oxidized lipids, as Lp[a] contains platelet-activating factor acetylhydrolase (PAF-AH) (119), an enzyme that inactivates PAF and oxidized lipids.

Whether Lp[a] is an acute-phase reactant is unclear. Some studies showed that levels of Lp[a] are increased during stress (120, 121), whereas others reported no changes or a reduction (122, 123). These conflicting data may be attributable to the specificity of the assays used to measure Lp[a] levels or to interindividual variation in plasma Lp[a] levels in the population.

HDL metabolism and decreased RCT. During infection and inflammation, there is a marked decrease in serum levels of HDL and apoA-I (16, 17, 27, 124). Furthermore, circulating HDL during infection, known as acute-phase HDL, has different characteristics from normal HDL. Acute-phase HDL is larger than normal HDL_3, its radius extending into the HDL_2 range, but it has a density comparable to that of HDL_3 (125). Acute-phase HDL is depleted in cholesteryl ester but enriched in free cholesterol, TG, and free FAs (24, 25, 27, 125–127). The phospholipid content of acute-phase HDL was increased in some studies (24,

27) but decreased in others (124, 125). In patients who underwent bypass surgery, acute-phase HDL had the same phospholipid-neutral lipid ratio, a decrease in phosphatidylethanolamine and phosphatidylinositol, and an increase in isoprostane-containing phosphatidylcholine and lysophosphatidylcholine (LPC) (127). In humans, there was a decrease in HDL sphingomyelin content (127), but an increase was observed in hamsters (128).

The hallmark of acute-phase HDL is an increase in apoSAA (24, 124, 125, 129, 130) and a decrease in apoA-I content (24, 124, 127, 130) (**Table 3**). The content of apoA-II and apoCs is decreased (24, 124, 130, 131), whereas apoE is found to be increased in some studies (24, 132) but decreased in others (130). HDL-associated apoJ is increased during inflammation and infection in rodents and humans (133–135). In contrast, several other proteins, including LCAT (24, 25, 136), cholesteryl ester transfer protein (CETP) (137, 138), hepatic lipase (HL) (139), and paraoxonase 1 (PON1) (134, 140), are decreased during the APR. The activity of HDL-associated plasma PAF-AH is acutely increased during inflammation in several rodent species (141), but a late decrease has also been reported in rabbits and mice (134, 135). Phospholipid transfer protein (PLTP) is decreased in rats injected with LPS (142), but data in humans are conflicting (132, 143). Finally, secretory phospholipase A_2 ($sPLA_2$), a phospholipase enzyme that hydrolyzes phospholipids in HDL, and LPS-binding protein (LBP) are markedly induced during infection and inflammation (144). SAA-rich HDL particles that are devoid of apoA-I have also been reported (145). We recently found that apoA-IV and apoA-V levels are increased in acute-phase HDL (our unpublished observations).

Although it is well established that infection and inflammation are associated with a reduction in serum HDL and apoA-I levels, the exact mechanism has not yet been established. Because apoSAA can displace apoA-I from HDL (146, 147) and apoSAA-rich HDL particles are rapidly cleared from the circulation (148), it has been assumed that the several-fold increase in apoSAA content in HDL is the mechanism for the decrease in apoA-I and HDL levels. However, we have shown that the decrease in HDL is very rapid, occurring before the increase in SAA (136). Furthermore, a study in mice in which apoSAA levels were markedly increased to levels comparable to those seen in infection found no changes in HDL cholesterol or apoA-I levels (149). Thus, high levels of SAA per se do not decrease HDL or apoA-I levels in the absence of the other changes that occur during infection and inflammation.

An increase in $sPLA_2$ has also been proposed to contribute to the reduction in HDL during infection/inflammation. Mice overexpressing $sPLA_2$ have reduced HDL concentrations (150), and HDL from these mice is catabolized more rapidly than HDL from normal mice (151). Although apoSAA is known to activate $sPLA_2$, overexpression of SAA in addition to $sPLA_2$ does not cause a greater reduction in the levels of HDL or apoA-I (152), further suggesting that the reduction of HDL during infection is not caused by an increase in apoSAA.

Endothelial lipase (EL) has been shown to regulate HDL metabolism (153–155). EL is synthesized by the endothelial cells and possesses phospholipase A-I activity. Overexpression of EL reduces HDL cholesterol levels (153), whereas inhibition of EL increases HDL levels (156). Treatment of cultured endothelial cells with TNF-α or IL-1β has been shown to increase the expression of EL (157). If similar effects occur in vivo, it may provide another mechanism for the reduction in HDL levels during infection.

The decrease in LCAT activity during infection may decrease HDL cholesterol levels caused by impaired esterification, similar to what is found in humans or animals with mutations in the LCAT gene (158). The decrease in HL may reduce pre-β HDL generation. Moreover, TG enrichment of HDL during infection may lead to the rapid clearance of apoA-I (159). Which of these changes contributes to the reduction of HDL and apoA-I during the APR is not yet established, but none accounts for the early decrease.

TABLE 3. Changes in proteins involved in HDL metabolism during infection and inflammation

Proteins	Effects
Increased	
Apolipoprotein serum amyloid A	Decreases cholesterol uptake by hepatocytes; increases cholesterol uptake into macrophages
Secretory phospholipase A_2	Decreases phospholipid content of HDL and impairs cholesterol removal from cells
ApoJ	Not known
PAF-AH	Increases lysophosphatidylcholine production
LPS binding protein	Increases neutralization of endotoxin by HDL
ApoE	Increases cholesterol delivery to cells; redirects endotoxin from macrophages to hepatocytes
ApoA-IV	Decreases endotoxin-induced stimulation of monocytes
ApoA-V	Not known
Ceruloplasmin	Enhances LDL oxidation
Decreased	
ApoA-I	Impairs cholesterol removal from cells
ApoA-II	Not known
LCAT	Impairs cholesterol removal from cells
CETP	Impairs cholesterol transfer to apoB-containing lipoproteins
Hepatic lipase	Decreases pre-β HDL generation
Paraoxonase 1	Decreases the ability of HDL to protect against LDL oxidation
Transferrin	Decreases the ability of HDL to protect against LDL oxidation

apoJ, apolipoprotein J; CETP, cholesteryl ester transfer protein; PAF-AH, platelet-activating factor acetylhydrolase.

HDL metabolism is tightly linked to RCT, a process by which cholesterol is removed from peripheral cells and transported to the liver for metabolism and/or excretion (160, 161). Several HDL-associated proteins and a number of cell surface receptors play a key role in RCT (**Fig. 4**). ApoA-I on HDL and ABCA1 in the plasma membrane are required for apolipoprotein-mediated cholesterol efflux. Subsequently, LCAT, which converts free cholesterol on HDL into cholesteryl ester, assists in cholesterol efflux by an aqueous diffusion mechanism. CETP then mediates the exchange of cholesteryl ester in HDL for TG in TG-rich lipoproteins. PLTP transfers phospholipids from TG-rich lipoproteins into HDL and promotes the remodeling of HDL. HL hydrolyzes TG and phospholipids in large α-HDL, generating small pre-β HDL particles that are efficient acceptors of cholesterol from plasma membrane. In the liver, scavenger receptor class B type I (SR-BI) plays a key role in the selective uptake of cholesteryl ester, whereas the β-chain of ATP synthase mediates endocytosis of HDL particles.

During infection and inflammation, there is a reduction in RCT attributable to multiple changes at each step in the pathway (Fig. 4). ABCA1 mRNA and protein levels in macrophages are decreased by LPS and cytokines (117, 162), impairing cholesterol efflux from cells. The decreases in apoA-I, HDL, and LCAT impair the acceptance of cellular cholesterol (163). The decrease in CETP activity limits the transfer of cholesteryl ester to TG-rich lipoproteins, further retarding the RCT pathway (138). HL activity is decreased (139), which would reduce the generation of pre-β HDL particles. In addition, during the APR, mRNA expression and protein levels of SR-BI in the liver are markedly decreased, which is accompanied by decreased cholesteryl ester uptake into hepatocytes (164). Therefore, during infection and inflammation, RCT is affected at the level of cholesterol removal from cells, transfer among particles, and uptake by the liver.

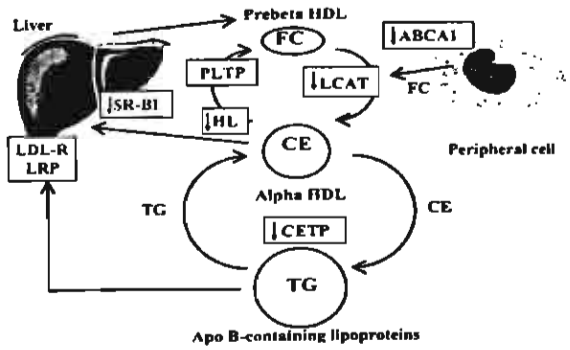

Fig. 4. Changes in reverse cholesterol transport during the APR. LPS and cytokines decrease ABCA1 and cholesterol efflux from peripheral cells to HDL. LPS also decreases several enzymes involved in HDL metabolism, including LCAT, cholesteryl ester transfer protein (CETP), and hepatic lipase (HL). In addition, LPS and cytokines downregulate hepatic scavenger receptor class B type I (SR-BI), resulting in a decrease in cholesteryl ester (CE) uptake into the liver. FC, free cholesterol; LDL-R, LDL receptor; LRP, LDL receptor related protein; PLTP, phospholipid transfer protein.

Sphingolipid metabolism

Sphingolipids such as ceramide and sphingomyelin are important constituents of plasma membranes. Glycosphingolipids (GSLs) are complex sphingolipids that contain a hydrophobic ceramide moiety and a hydrophilic oligosaccharide residue. Both sphingolipids and GSLs are components of plasma lipoproteins and are involved in several biological processes, including cell recognition and proliferation, signal transduction, interaction with bacterial toxins, and modulation of the immune response.

The metabolism of sphingolipids and GSLs is altered during infection and inflammation. LPS stimulates hepatic ceramide and sphingomyelin synthesis by increasing the mRNA expression and activity of serine palmitoyltransferase (SPT), the first and rate-limiting enzyme in sphingolipid synthesis that catalyzes the condensation of serine with palmitoyl-CoA (128) (**Fig. 5**). LPS increases the transcription rate, mRNA expression, and activity of glucosylceramide (GlcCer) synthase, the first committed enzyme in the GSL synthesis pathway, in the liver (165). GlcCer is the precursor of all neutral GSLs as well as sialic acid-containing acidic GSLs or gangliosides. The LPS-induced increase in GlcCer expression occurs earlier than the increase in SPT mRNA levels. It is possible that the increase in hepatic GlcCer production during the APR is the primary event, which then signals for more substrate, resulting in the induction of SPT and subsequent increase in ceramide synthesis. This hypothesis is supported by the fact that steady-state levels of GlcCer and its distal metabolites, including ceramide trihexoside and ganglioside GM3, are increased in the liver after LPS treatment (165), whereas in contrast, the content of ceramide, the substrate for GlcCer synthesis, is decreased in the liver despite the increase in SPT (165). Like LPS, TNF and IL-1 also increase both SPT and GlcCer mRNA expression in the

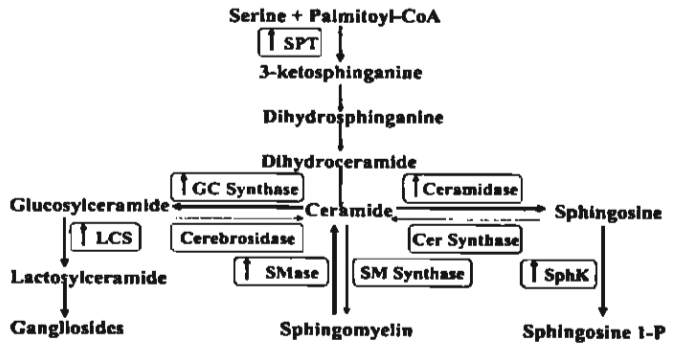

Fig. 5. Changes in sphingolipid metabolism during the APR. LPS and cytokines stimulate ceramide (Cer) and sphingomyelin (SM) synthesis in the liver by increasing the expression and activity of serine palmitoyltransferase (SPT), the rate-limiting enzyme in sphingolipid synthesis. LPS also increases the activity of glucosylceramide (GC) synthase, the first committed enzyme in the glycosphingolipid synthesis pathway. As a result, lipoproteins are enriched with ceramide, sphingomyelin, and glycosphingolipids. In addition, LPS and cytokines increase the activity of secretory sphingomyelinase (SMase) in the serum, resulting in increased levels of ceramide in serum. 1-P, 1-phosphate.

liver, suggesting that these cytokines mediate the LPS effect (128, 165).

Likely as a consequence of the LPS-induced increase in hepatic sphingolipid synthesis, all lipoprotein fractions isolated from LPS-treated animals contain significantly higher levels of ceramide, sphingomyelin, and GlcCer (128). An increase in ceramide content in LDL may enhance the susceptibility of LDL toward aggregation.

LPS also upregulates the mRNA expression and activities of SPT and GlcCer synthase in extrahepatic tissues, including spleen and kidney (166). The content of ceramide in spleen or kidney, however, is not increased, suggesting that newly synthesized ceramide is used as a substrate to increase GlcCer synthesis (166). Specific GSLs are ligands for a T-cell receptor expressed on natural killer T-lymphocytes, and GSLs stimulate the proliferation of specific subsets of lymphocytes (167). One can speculate that the LPS-induced increase in GSL content of these tissues is used to regulate cellular proliferation and modulate the immune response.

In addition to activating the enzymes that synthesize sphingolipids and GSLs, LPS and cytokines also induce enzymes involved in the hydrolysis of sphingolipids (Fig. 5). Treatment with LPS, TNF, or IL-1 acutely increases the serum activity of secretory sphingomyelinase (168). Serum ceramide levels are increased in animals treated with LPS and in patients with sepsis (128, 169, 170). The APR also activates ceramide-metabolizing enzymes. IL-1 activates both neutral and acid ceramidases in cultured rat hepatocytes, resulting in increased formation of sphingosine (171), whereas in cultured endothelial cells, TNF induces sphingosine kinase activity and increases the formation of sphingosine-1-phosphate (172). These studies suggest that several enzymes involved either in the de novo synthesis of ceramide and its downstream metabolites or in the hydrolysis of ceramide are induced by LPS and cytokines. Because ceramide and its metabolites are involved in signal transduction and cellular regulation, particularly in cells of the immune system, it makes sense that several anabolic and catabolic pathways of sphingolipid metabolism are induced during infection and inflammation to maintain a delicate balance between ceramide and its metabolites in the cell. Figure 5 summarizes the effects of LPS and APR-inducing cytokines on sphingolipid and GSL metabolism.

ROLE OF NUCLEAR HORMONE RECEPTORS IN THE REGULATION OF LIPID METABOLISM DURING INFECTION AND INFLAMMATION

Nuclear hormone receptors and lipid metabolism

Most, if not all, of the changes in lipid metabolism that are induced by infection and inflammation are attributable to changes in gene transcription (13). The mechanisms by which gene transcription is increased during the APR have been extensively studied. Class 1 positive acute-phase proteins are increased by IL-1-type cytokines, whereas the IL-6 family of cytokines increase class 2 positive acute-phase proteins (173, 174). Activation of nuclear factor κB (NF-κB) and nuclear factor interleukin-6 (NF-IL-6) mediates IL-1-stimulated increases in acute-phase protein transcription, whereas activation of NF-IL-6 and the Janus kinase-signal transducers and activators of transcription pathway mediates IL-6 family stimulation of acute-phase protein transcription (174). Much less is understood regarding the mechanism of the downregulation of transcription of negative acute-phase proteins during the APR, and many of the changes in lipid metabolism seen in infection and inflammation are mediated by decreases in proteins and their transcription (13).

Nuclear hormone receptors are a large family of transcription factors, characterized by a central DNA binding domain that targets the receptor to specific DNA sequences (response elements) and a C-terminal portion that includes a ligand binding domain, which recognizes specific hormones, vitamins, drugs, or other lipophilic compounds (175–178). Several nuclear hormone receptors, including the peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and farnesoid X receptor (FXR), bind and are activated by lipids (176–181). Furthermore, the increased activity of these receptors regulates the transcription of a large number of genes involved in multiple aspects of lipid and lipoprotein metabolism (182). Because of their abilities to sense intracellular lipid levels and orchestrate changes in lipid metabolism, these nuclear hormone receptors have been recognized as liposensors (178). Finally, these liposensors (PPARs, LXRs, and FXR) heterodimerize with retinoid X receptors (RXRs) for efficient gene regulation (175). As discussed in detail below, most of the genes of lipid metabolism that decrease during the APR are regulated by these liposensors and related transcription factors, and the downregulation of these liposensors plays a key role in those changes.

Regulation of liposensors during infection and inflammation

In hamsters and mice, LPS administration decreases both protein and mRNA levels of RXR-α, -β, and -γ in the liver (183) (**Table 4**). The decrease in RXR occurs rapidly (within 4 h) and is sustained. Administering TNF and IL-1 reproduces these LPS effects. Similar reductions in RXR isoforms are seen in Hep3B cells treated with TNF and IL-1 but not IL-6, indicating that the decreases are directly induced by the cytokines (M-S. Kim, J. K. Shigenaga, A. H. Moser et al., unpublished observations). Furthermore, LPS administration also significantly reduces the hepatic nuclear DNA-binding activity of RXR homodimers to an RXR response element (183).

In addition to inhibiting the expression of the obligate liposensor heterodimer partner RXR, LPS and cytokine administration also reduces hepatic mRNA levels of PPAR-α and -γ, LXR-α, FXR, pregnane X receptor (PXR), and constitutive androstane receptor (CAR) (183–185). These decreases were associated with reductions in nuclear binding activity to a direct repeat-1 (DR-1) PPAR response element, a DR-4 LXR response element, and an in-

TABLE 4. Changes in nuclear hormone receptors and their target genes involved in FA and TG metabolism during infection and inflammation

Tissue	Nuclear Receptor	Target Genes	Function
Adipocytes	PPAR-γ ↓	AP2 ↓	Fatty acid transport (intracellular)
		LPL ↓	TG catabolism
		FATP ↓	Fatty acid transport
		CD36/FAT ↓	Fatty acid and oxidized LDL uptake
		ACS ↓	Fatty acid esterification
Heart	PPAR-α ↓	LPL ↓	TG catabolism
	PPAR-β/δ ↓	FATP ↓	Fatty acid transport
		CD36/FAT ↓	Oxidized LDL uptake
		H-FABP ↓	Fatty acid transport (intracellular)
		CPT-Iβ ↓	Fatty acid oxidation
		ACS ↓	Fatty acid esterification
Skeletal muscle	PPAR-α ?	LPL ↓	TG catabolism
	PPAR-β/δ ?	FATP ↓	Fatty acid transport
		CD36/FAT ↓	Oxidized LDL uptake
		H-FABP ↓	Fatty acid transport (intracellular)
		ACS ↓	Fatty acid esterification
Liver	PPAR-α ↓	FATP ↓	Fatty acid transport
	PPAR-γ ↓	CD36/FAT ↓	Oxidized LDL uptake
		H-FABP ↓	Fatty acid transport (intracellular)
		CPT-Iα ↓	Fatty acid oxidation
	FXR ↓	ApoC-II ↓	Increases LPL activity
		ApoE ↓	Lipoprotein metabolism

ACS, acyl-CoA synthetase; AP2, adipocyte P2; CPT, carnitine palmitoyl transferase; FABP, fatty acid binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; FXR, farnesoid X receptor; H-FABP, heart-FABP; PPAR, peroxisome proliferator-activated receptor; ↓, decreased levels of mRNA after LPS treatment.

verted repeat-1 FXR response element (183, 184). In contrast, mRNA levels of PPAR-β/δ and LXR-β were not significantly altered in the liver after LPS treatment.

In adipose tissue, PPAR-γ levels decrease after the administration of LPS or TNF (186) (Table 4). Treatment of adipocytes in vitro with TNF, IFN-γ, and IL-11 decreases mRNA levels of PPAR-γ (187–191). The effect of LPS and cytokines on RXR isoforms and other liposensors in adipose tissue remains to be determined. In cardiac muscle, our laboratory recently reported that LPS administration decreases RXR-α, -β, and -γ and PPAR-α and -β/δ expression (192) (Table 4). To our knowledge, studies of the effect of inflammation and infection on the expression of RXR, PPAR, and other liposensors in skeletal muscle have not been carried out. Lastly, although the levels of liposensors are regulated in tissues that play a major role in the alterations of lipid metabolism during the APR, recent studies by our laboratory have shown that changes in the levels of RXR, PPARs, and LXRs were not found in the small intestine, an organ in which lipid metabolism is not significantly altered during infection and inflammation (117). Thus, liposensor levels specifically change in the tissues that exhibit changes in lipid metabolism during the APR.

Consequences of decreased expression of liposensors

Although it is likely that many factors influence the diverse changes in lipid and lipoprotein metabolism that occur in response to infection/inflammation, alterations in the activity of nuclear hormone receptor liposensors are likely to play a pivotal role in the coordinated regulation of FA and cholesterol metabolism that occurs during the APR, as can be seen by examining the effects on genes that liposensors are known to regulate.

FA and TG metabolism. As discussed earlier, infection/inflammation is characterized by an increase in lipolysis and a decrease in FA oxidation in adipose tissue, contributing to hypertriglyceridemia (26). PPAR-γ has been shown to directly regulate genes that promote the storage of fat in adipose tissue, including adipocyte P2, LPL, FATP, CD36/FAT, and ACS (179, 193, 194). As discussed above, during infection and inflammation the expression of these genes is decreased, and it is likely that the reduction in PPAR-γ activity in adipose tissue contributes to the changes in these proteins that would reduce fat storage and enhance lipolysis.

Likewise, downregulation of RXR-α, -β, and -γ and PPAR-α and -β/δ in cardiac muscle would be expected to reduce FA oxidation. Activation of PPAR-α and -β/δ induces the expression of many key enzymes required for FA oxidation, including LPL, FATP, CD36/FAT, heart-FABP (H-FABP), CPT-Iβ, and ACS (179, 195–198). One can postulate that a reduction in PPAR-α and -β/δ activity in the heart during the APR contributes to the decreased expression of these genes (67, 68, 71, 199) (Table 4). In skeletal muscle, there is also a decrease in FA oxidation, which is associated with a decrease in LPL, FATP, CD36/FAT, H-FABP, and ACS (67, 68, 71, 200). Whether levels of RXR-α, -β, and -γ and PPAR-α and -β/δ change in skeletal muscle during the APR remains to be determined.

Downregulation of RXR-α, -β, and -γ and PPAR-α and -γ in the liver during the APR could also reduce hepatic FA oxidation, as a number of key PPAR-regulated proteins required for FA oxidation are decreased, including FATP, CD36/FAT, liver-FABP, and CPT-Iα (ACS is decreased in mitochondria but not in endoplasmic reticulum) (67, 68, 70, 71) (Table 4). In contrast, many proteins involved in the reesterification of FA and the secretion of VLDL

from the liver are not decreased and are not regulated by the PPARs.

Decreased hepatic FXR activity could also contribute to the increase in serum TGs during infection (184). FXR-deficient mice have increased serum TG levels (201), and FXR has been shown to regulate the hepatic expression of apoC-II and apoE (202, 203), both of which are decreased during the APR (85, 184).

Regulation of gene transcription is complex, involving multiple transcription factors. Therefore, changes in PPARs, FXR, and RXR are unlikely to be the only transcription factors that regulate the genes of interest during the APR. For example, Berg, Calnek, and Grinnell (204, 205) have shown that IL-1- and IL-6-induced decreases in apoE mRNA levels in HepG2 cells are associated with the phosphorylation of BK virus enhancer factor-1, a member of the NF-1 family of nuclear factors, to its isoform B1. An increase in B1 is associated, by unknown mechanisms, with decreases in apoE mRNA levels (205). Thus, an increase in the B1 isoform coupled with the reduction in FXR activity during infection and inflammation may together result in the decrease in apoE expression. Likewise, we recently found that PPAR-γ coactivating factor-1 (PGC-1), which interacts with PPAR-α, PPAR-γ, hepatocyte nuclear factor-4 (HNF-4), and other nuclear hormone receptors, is reduced during the APR (M-S. Kim, J. K. Shigenaga, A. H. Moser, et al., unpublished observations).

Thus, decreases in RXR, PPARs, LXR, and related transcription factors in adipose tissue, muscle, and liver could be mechanisms by which the characteristic changes in TG and FA metabolism that occur during infection and inflammation are induced.

RCT. RCT is a complex process that involves transporters in peripheral tissues and liver, enzymes and transfer proteins in the serum, receptors in the liver, the synthesis of bile acids in the liver, and the secretion of cholesterol and bile acids into the bile (160, 161). Many of the proteins essential for RCT are regulated by liposensors (181), whose changes could mediate the reduction in RCT that occurs during infection and inflammation.

PERIPHERAL TISSUES. ABCA1 transporters play a dominant role in the movement of cholesterol from cells to HDL and are regulated by LXR (206–208). Treatment of macrophages with LPS or cytokines decreases ABCA1 (117, 162). However, no change in RXR or LXR that could account for the reduction in ABCA1 expression was found in macrophages (117, 209) (**Table 5**). Recently, bacterial infection was found to activate Toll-like receptor 4, inhibiting the induction of LXR target genes, including ABCA1 (209). This cross-talk between LXR and Toll-like receptor signaling decreases cholesterol efflux from macrophages (209). In addition, LPS-induced decreases in the expression of CYP27A1 would decrease the production of 27-hydroxycholesterol, a likely endogenous ligand of LXR, further explaining the effect of LPS on LXR target genes (110).

ENZYMES AND TRANSFER PROTEINS IN THE SERUM. CETP mediates the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins (210). CETP expression is regulated by LXR activity (211); decreased RXR/LXR activity in the liver likely contributes to the reduced CETP expression seen during the APR. PLTP mediates the transfer of phospholipids and cholesterol from TG-rich lipoproteins to HDL. PLTP expression in the liver is regulated by FXR activity (212); decreased RXR/FXR activity in the liver could contribute to the reduction in hepatic PLTP expression during the APR (142) (Table 5).

RECEPTORS IN THE LIVER. SR-BI mediates the selective uptake of cholesteryl esters from HDL into the liver (213). PPARs and FXR regulate the expression of SR-BI (214, 215). Therefore, the decrease in PPAR/RXR and FXR/RXR activity in the liver could mediate the decrease in SR-BI expression during the APR (Table 5).

TABLE 5. Changes in nuclear hormone receptors and their target genes involved in reverse cholesterol transport during infection and inflammation

Tissue	Nuclear Receptor	Target Genes	Function
Macrophage	LXR ↔	ABCA1 ↓	Cholesterol efflux
Liver	LXR ↓	CETP ↓	Cholesteryl ester transfer
		ABCG5/ABCG8 ↓	Cholesterol and phytosterol efflux
		CYP7A1 ↓	Bile acid synthesis
	FXR ↓	PLTP ↓	Phospholipid transfer
		MDR-2 ↓	Phospholipid secretion
		SHP ↓	Inhibits bile acid synthesis
		BSEP ↓	Canalicular bile salt excretion
		SR-BI ↓	Cholesteryl ester uptake
	PPAR-α ↓	MDR-2 ↓	Phospholipid secretion
	PXR ↓	MDR-2 ↓	Phospholipid secretion
	LRH-1 ↓	CYP7A1 ↓	Bile acid synthesis
		CYP8B1 ↓	Cholic acid synthesis
	HNF-4 ↓	CYP8B1 ↓	Cholic acid synthesis
	HNF-1* ↓	CYP27A1 ↓	Bile acid synthesis

BSEP, bile salt export pump; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, sterol 27-hydroxylase; HNF, hepatocyte nuclear factor; LRH-1, liver receptor homolog-1; LXR, liver X receptor; MDR-2, multidrug resistance-2; PLTP, phospholipid transfer protein; PXR, pregnane X receptor; SHP, small heterodimer partner; SR-BI, scavenger receptor class B type I; ↔, unchanged; ↓, decreased.

* Not a nuclear hormone receptor.

HEPATIC SYNTHESIS OF BILE ACIDS. CYP7A1 is the key rate-limiting enzyme in the neutral bile acid synthetic pathway (105). CYP7A1 is regulated by LXR in rodents and FXR in rodents and humans (216–219). In contrast, increases in FXR/RXR activation reduce CYP7A1 activity by increasing small heterodimer partner (SHP), which in turn blocks the ability of the transcription factor liver receptor homolog-1 (LRH-1), to stimulate CYP7A1 expression (220).

During infection/inflammation, RXR, LXR, FXR, SHP, LRH-1, and other transcription factors decrease, with the net result being a decrease in CYP7A1 activity, despite the decreases in FXR and SHP (183, 184). There are several possible explanations for the decrease in CYP7A1 activity during the APR. First, LXR/RXR activation may be a dominant factor in regulating the transcription of CYP7A1 (221); hence, the reduction in LXR/RXR activity may result in decreased CYP7A1 expression. Second, the decrease in FXR/RXR activity and SHP may not be crucial in the complex setting of inflammation; their decrease would normally result in an increase in the activity of LRH-1, but during the APR LRH-1 is independently reduced (184), thereby decreasing CYP7A1. Lastly, expression of CYP7A1 is regulated by a number of other transcription factors, such as HNF-4, PXR, and thyroid hormone receptor (TR) (222); our laboratory and others have shown that these transcription factors are also downregulated during the APR (185, 223, 224). Thus, multiple factors may produce the decrease in CYP7A1 expression (Table 5).

Sterol 12α-hydroxylase (CYP8B1) is an enzyme in the bile synthetic pathway responsible for cholic acid synthesis (105). Unpublished studies by our laboratory have shown that mRNA levels of CYP8B1 decrease after LPS administration. Two key transcription factors that increase the expression of CYP8B1 are LRH-1 and HNF-4 (225, 226), both of which are decreased during the APR (184, 223, 227), which could account for the decrease in CYP8B1 mRNA (Table 5).

During the APR, expression of CYP27A1, a key enzyme in both the classic and alternative pathways of bile acid synthesis, is decreased (110). HNF-1, the transcription factor regulating the expression of CYP27A1 (228), is decreased during the APR (110, 227, 229), which could account for the changes (Table 5). HNF-4 stimulates the expression of HNF-1; the decrease in HNF-4 that occurs in the APR could explain the decrease in HNF-1.

SECRETION OF CHOLESTEROL AND BILE ACIDS INTO THE BILE. As discussed above, the secretion of bile acids into the bile is mediated by BSEP and MRP2, the secretion of cholesterol is mediated by ABCG5/ABCG8, and the secretion of phospholipids is mediated by MDR2 (106, 107). Expression of these transporters is regulated by liposensors. Specifically, FXR activation increases BSEP expression (230), FXR, PXR, and CAR activation increase MRP2 expression (231), LXR activation increases ABCG5 and ABCG8 expression (232), and PPAR-α activation increases MDR2 expression (233). Thus, the decreases in FXR, LXR, PPAR-α, PXR, and CAR during the APR (183–185) are likely to contribute to decreases in these transporters and to decreased secretion of lipids into the bile (Table 5).

As summarized in Tables 4 and 5, these data demonstrate that the reduction in the nuclear hormone liposensors (PPARs, LXR, and FXR) could account for many of the changes in lipid and lipoprotein metabolism that occur during infection and inflammation. However, we have also shown that changes occur in several related transcription factors, and it is likely that other transcription factors are also involved in the complex regulation that occurs during the APR. Lastly, as additional regulatory functions of PPARs, LXR, FXR, RXR, PXR, CAR, and TR are recognized, the decrease in these nuclear hormone receptors may be shown to mediate other changes in metabolism that occur during the APR, such as changes in glucose, bilirubin, steroid hormone, and drug metabolism.

PROATHEROGENIC CHANGES IN LIPID AND LIPOPROTEIN METABOLISM DURING INFECTION AND INFLAMMATION

The forgoing has demonstrated that during the course of infection and inflammation, a multitude of changes occur in the structure, composition, and function of lipoproteins. Many of these changes in lipoproteins are similar to those proposed to promote atherogenesis. Several epidemiological studies have suggested that the risk and/or incidence of coronary artery disease (CAD) is higher in patients with infections and/or chronic inflammatory diseases (234–237). Some studies have suggested that specific infectious agents, such as *Chlamydia pneumoniae* and cytomegalovirus, play a direct role in the vessel wall in the formation of atherosclerotic lesions (238, 239). However, the prevalence of CAD is also higher in patients with *Helicobacter pylori* infection, chronic dental infection, chronic urinary tract infections, and chronic bronchitis, infections in which the microorganisms are not localized to the vessel wall (240–242, 242a). The presence of circulating endotoxin also predicts future atherosclerosis (242a). Finally, there is an increased incidence of CAD in patients with inflammatory diseases such as rheumatoid arthritis, psoriasis, and systemic lupus erythematosus (243–246). Although all of these infections and inflammatory conditions have a distinct etiological origin, they are associated with a common, sustained systemic APR. In addition, more common diseases that predispose to atherosclerosis, such as diabetes, obesity, and metabolic syndrome, are also associated with inflammation (6–10). We have proposed that the APR-associated structural and functional changes in lipoproteins could be one possible link between infection/inflammation and atherosclerosis (14). Because atherosclerosis itself is an inflammatory disease and inflammation causes proatherogenic changes in lipoproteins, a vicious cycle could develop, resulting in worsening of atherosclerosis.

VLDL metabolism

Evidence is accumulating that TG-rich lipoproteins are proatherogenic (247–249). VLDLs from hypertriglyceri-

demic individuals are toxic to endothelial cells (250). They can interact with LDL receptors and receptors for apoB-48 on the monocytes/macrophages, resulting in enhanced lipid uptake and foam cell formation (251). VLDLs secreted by the liver after LPS administration are also enriched in sphingolipids (128). Because sphingomyelin enrichment can decrease the clearance of TG-rich lipoproteins (252), the increase in VLDL sphingolipids during infection and inflammation can result in the accumulation of atherogenic remnant particles. Thus, the APR-associated changes in TG and VLDL metabolism can be proatherogenic.

LDL metabolism

Although circulating levels of total and LDL cholesterol in humans decrease during infection, other changes in LDL metabolism could promote atherogenesis. In patients with acquired immune deficiency syndrome (AIDS), a decrease in LDL levels is associated with a decrease in particle size, resulting in small dense LDLs (subclass pattern B) (253). These LDL particles are more proatherogenic because they have a lower binding affinity for the LDL receptor, which leads to impaired clearance and increased circulation time for these particles (254). Moreover, small dense LDLs can cross the endothelial barrier more effectively and bind to proteoglycans in the vascular wall intima, resulting in LDL retention (255). Additionally, small dense LDLs are more susceptible to oxidative modifications, resulting in rapid uptake and cholesterol accumulation in the macrophages (256). The increase in small dense LDLs is likely the consequence of hypertriglyceridemia during infection (253).

Oxidative modification of LDL plays a central role in the pathogenesis of atherosclerosis (257). We have shown that the levels of several markers of lipid peroxidation, including conjugated dienes, thiobarbituric acid-reactive substances, lipid hydroperoxides, and LPC, are increased in serum and/or circulating LDL in animals treated with LPS (258). Moreover, LDL isolated from LPS-treated animals is more susceptible to oxidation in vitro (258). Children with infection have increased antibodies to oxidized LDL, and their LDL may be more susceptible to further oxidation in the vessel wall (259).

CRP is a classic acute-phase protein that binds phosphorylcholine residues of phospholipids or microbial products (260). CRP is associated with VLDL and LDL and is present in atherosclerotic lesions (261). High levels of CRP have been shown to be an independent risk factor for CAD, which is thought to represent the inflammatory nature of atherosclerosis (262). CRP binds oxidized LDL and oxidized phospholipids, which then enhances uptake by macrophages (263), promoting the formation of foam cells using the oxidized LDL.

During infection and inflammation, increases in $sPLA_2$ are likely to promote atherosclerosis. $sPLA_2$ hydrolyzes phospholipids in LDL at the *sn*-2 position, generating polyunsaturated FAs that can be oxidized (144). These oxidized FAs can further modify LDL to yield oxidized LDL. In addition, $sPLA_2$-induced lipolysis of LDL phospholipid increases LDL particle fusion and enhances LDL binding to proteoglycans (264), both of which promote atherogenesis. Transgenic mice expressing human $sPLA_2$ exhibit significant atherosclerosis even when maintained on a low-fat diet (265).

The protein and lipid composition of LDL particles is altered during infection/inflammation. In humans, the majority of plasma PAF-AH activity is associated with LDL, whereas in rodents, most plasma PAF-AH activity is found on HDL (266). Plasma PAF-AH degrades PAF, a proinflammatory phospholipid mediator produced during infection and inflammation. However, PAF-AH also hydrolyzes lipoprotein-associated phosphatidylcholine, generating LPC, a molecule that exerts several proatherogenic effects (267–269). During the LPS and cytokine-induced APR, there is an acute increase in plasma and HDL-associated PAF-AH activity in several rodent species (141). Moreover, in patients with chronic human immunodeficiency virus (HIV) infection, plasma PAF-AH activity is increased, mainly in LDL (270). There is also a marked increase in the LPC content of circulating LDL in animal models of infection (258). In humans, circulating levels of PAF-AH are a strong and independent risk factor for CAD (271). Thus, increased plasma PAF-AH activity during the APR could have proatherogenic consequences.

Circulating LDL is more enriched in several sphingolipids, including sphingomyelin, ceramide, and GlcCer, during infection/inflammation (27, 128). Sphingolipid enrichment may increase the atherogenic potential of LDL, as LDL isolated from atherosclerotic lesions is enriched in sphingomyelin, ceramide, and GlcCer (272, 273). Plasma sphingomyelin levels are also increased in animal models of atherosclerosis and in humans with CAD (274, 275). When sphingomyelin on LDL is delivered into the arterial wall, it can be partly converted into ceramide by an arterial wall sphingomyelinase. Because LPS and cytokines increase the circulating levels of secretory sphingomyelinase (168), they may enhance the production of ceramide; ceramide promotes lipoprotein aggregation, stimulating LDL uptake by macrophages (276). Similarly, ceramide-rich LDL extracted from atherosclerotic lesions is either aggregated or has an increased tendency to aggregate (272). Thus, the various sphingolipids that are increased during the APR enhance the atherogenicity of lipoproteins in multiple ways.

In summary, during infection/inflammation several changes occur in LDL, such as the generation of small dense LDLs, increased susceptibility toward oxidation, increased CRP, $sPLA_2$-induced hydrolysis of LDL phospholipids, high plasma PAF-AH activity, and LDL enrichment with TG, cholesterol, LPC, and sphingolipids. These alterations change the structure and function of LDL, rendering it more proatherogenic.

HDL metabolism

Many changes in HDL metabolism occur during infection/inflammation that can impair the antiatherogenic functions of HDL. As discussed above, several HDL-associated proteins involved in the RCT pathway are decreased,

including apoA-I, LCAT, CETP, HL, and SR-BI (Table 3). During the APR, cholesterol removal from cells is decreased (163, 277, 278) as a result of a reduction in LCAT in acute-phase HDL (163). Moreover, cholesteryl ester delivery to hepatocytes is decreased as a result of a decrease in SR-BI (164, 279). Although an initial decrease in RCT during the APR may be beneficial as it redirects cholesterol toward macrophages for host defense (see below), a prolonged or sustained APR, as seen in chronic infection and inflammation, may continually impair RCT, thus leading to cholesterol deposition in macrophages and promoting atherogenesis.

Another key physiological function of HDL is protecting LDL against oxidation. Several HDL-associated proteins, including PON1, PON3, ceruloplasmin, transferrin, and apoA-I, possess antioxidant activity. Their removal or inactivation increases the susceptibility of LDL toward oxidation (280, 281), although the in vivo contribution of each is not yet established. During infection and inflammation, HDL loses its antioxidant function and becomes prooxidant (134, 135).

PONs constitute a group of enzymes that hydrolyze phospholipids with longer acyl chains and are capable of protecting LDL against oxidation in vitro. Depletion of PON1 results in the loss of antioxidant function of HDL, and addition of PON1 restores the protective function of HDL (134). Lipoproteins isolated from PON1-deficient mice are more susceptible to oxidation than lipoproteins isolated from their wild-type littermates, and PON1-deficient mice are more susceptible to atherosclerosis, suggesting that PON1 plays a role in preventing lipoprotein oxidation and atherogenesis (281). Acute-phase HDL has lower PON1 activity and is unable to protect LDL against in vitro oxidation (134). Moreover, during the LPS- and cytokine-induced APR, hepatic PON1 mRNA expression and serum PON1 activity decrease (134, 140), which precede the appearance of oxidized LDL (258), raising the possibility that the decreased PON1 activity during the APR contributes to the increased LDL oxidation in vivo.

Levels of two other HDL-associated proteins, ceruloplasmin and transferrin, change during infection and could contribute to increased LDL oxidation. Ceruloplasmin is a copper binding protein whose levels increase during the APR (282). Ceruloplasmin increases LDL oxidation in cell-free systems as well as in cultured cell lines, suggesting a prooxidant role (283, 284). In contrast, transferrin levels decrease during infection (285). Transferrin, which binds iron, may be antioxidant, as removal of HDL particles that contain transferrin activity reduces the ability of HDL to protect against LDL oxidation (280). Thus, three independent changes in HDL-associated proteins, a decrease in PON activity, an increase in ceruloplasmin, and a decrease in transferrin, could deplete HDL of its antioxidant function during the APR, converting HDL into a prooxidant, proinflammatory, and proatherogenic lipoprotein that is compounded by its decreased effectiveness in RCT, enhancing the atherogenic process (Table 3).

There are also direct effects of infection on macrophages, which could increase the risk of atherosclerosis. LPS and cytokines (TNF and IL-1) activate macrophages to accumulate lipids (286–288). LPS-stimulated macrophages accumulate more TGs and cholesteryl ester from lipoproteins than do unstimulated cells. *Chlamydia pneumoniae* infection of human-derived macrophages induces foam cell formation in the presence of LDL (289). Therefore, synergistic changes in lipoproteins and host cells during infection and inflammation could promote atherogenesis.

BENEFICIAL EFFECTS OF CHANGES IN LIPID AND LIPOPROTEIN METABOLISM DURING INFECTION AND INFLAMMATION

We have proposed that the changes in lipid and lipoprotein metabolism that occur during the host response to infection/inflammation include antiinfective and antiinflammatory effects that contribute to the host defense (13). Indeed, there is ample evidence that lipoproteins are part of innate immunity, the immediate protection against infection and inflammation. Below, we discuss these actions of lipoproteins with reference to changes that occur in the APR.

Lipoproteins and bacterial endotoxin

A humoral component other than antibody and complement was initially found to inactivate LPS in serum (290, 291). Subsequent studies have shown that lipoproteins, including HDL, chylomicrons, VLDL, LDL, and Lp[a], have the ability to bind and neutralize LPS in vitro (292–300). In addition, lipoproteins can bind LTA and α-toxin from *Staphylococcus aureus* (301, 302). When purified LPS was added to normal human whole blood in vitro, the majority of LPS was detected in HDL (60%), followed by LDL (25%) and VLDL (12%) (303). Similar results were found with LTA (304). However, during sepsis, when HDL levels decrease, LPS binding shifts to VLDL (305, 306). Isolation of plasma lipoproteins from normal healthy volunteers using strict apyrogenic techniques found LPS associated with VLDL, suggesting that the interaction between lipoproteins and LPS may be operative in vivo and is not simply attributable to contamination during isolation (295). The use of different anticoagulants for plasma preparation (e.g., heparin vs. EDTA) affects the distribution of LPS among classes of lipoproteins (307).

Binding of LPS to lipoproteins protects animals from LPS-induced fever, hypotension, and death (292, 293, 295, 308). Infusion of reconstituted HDL protects against endotoxic shock and gram-negative bacteremia in rabbits (309–311). Improved survival occurs when infusions of chylomicron or synthetic TG-rich lipid emulsion were given to animals up to 30 min after LPS, indicating that lipoproteins may have a therapeutic role during endotoxemia (312). Additionally, TG-rich lipoproteins protect rats from death when gram-negative sepsis is induced by cecal ligation and puncture (313).

Further evidence of lipoprotein protection comes from models of hypolipidemia or hyperlipidemia. Hypolipidemic rats, produced by 4-aminopyrolo-(3,4-D)pyrimide

(which prevents the hepatic secretion of lipoproteins) or estradiol (which increases hepatic receptors, leading to increased lipoprotein clearance), are more sensitive to LPS-induced lethality (314). Administration of exogenous lipoproteins to these hypolipidemic rats, increasing serum lipid concentrations into the physiological range, reverses the increased mortality to levels similar to those of control animals. In contrast, transgenic mice overexpressing apoA-I, which have high HDL levels, and LDL receptor-deficient mice, which have high LDL levels, are resistant to LPS-induced lethality and severe gram-negative infections (315, 316).

Taken together, these animal studies provide strong evidence that circulating lipoproteins play a vital role in host defense during endotoxemia. Increasing lipoprotein levels may be a viable therapeutic strategy to block or neutralize the toxic effects of LPS. Although the LPS-binding capacity of lipoproteins is 10- to 1,000-fold above the maximal concentrations of LPS observed in patients with sepsis, it is not sufficient to inhibit the effects of LPS during massive infection (315). In the circulation, LPS binds and activates monocytes more rapidly than lipoprotein binding and neutralization occur. However, an increase in the lipoprotein/LPS molar ratio, as occurs during infusion of lipoproteins, can accelerate the kinetics of the neutralization of LPS, providing some advantage (317).

Lipoproteins protect against harmful effects of LPS in humans. Reconstituted HDL decreases flu-like symptoms, changes in leukocyte counts, and cytokine release during endotoxemia (318). When LPS was preincubated with fasting or hypertriglyceridemic whole blood, the majority of LPS was bound to lipoproteins and the host response to LPS was attenuated (319). However, when LPS was infused into the circulation without preincubation, the interaction between leukocytes and LPS was favored. As a result, TG-rich fat emulsions could not inhibit the inflammatory response to LPS in humans (320).

Several potential mechanisms for the protective effect of lipoproteins against LPS have been found. When lipoprotein-bound LPS is injected into animals, the fate of LPS is altered. LPS bound to chylomicrons is cleared more rapidly than LPS alone (308). When LPS enters the circulation, the liver is the primary site of clearance; LPS is primarily taken up by hepatic macrophages (Kupffer cells), which are activated and secrete cytokines. Although cytokines play a role in host defense, high levels of cytokine secretion are the cause of septic shock. However, binding of LPS by lipoproteins decreased uptake by hepatic macrophages and increased uptake by hepatocytes, resulting in rapid secretion of LPS into the bile (308, 312, 321). Consistent with these findings, circulating levels of TNF were lower (308). Uptake of chylomicron-bound LPS into hepatocytes is also associated with the selective inhibition of NF-κB, a mediator of LPS activation (322).

Similarly, in vitro studies demonstrate that lipoproteins can prevent the activation of peripheral monocytes/macrophages by LPS, decreasing cytokine synthesis and secretion (323–327). Additionally, infusion of HDL reduces CD14 expression on monocytes (318). Once LPS is bound to monocytes, lipoproteins have been shown to promote the release of LPS from the cell surface, further attenuating the cellular response to LPS (328). Collectively, these studies suggest that lipoproteins can help neutralize the lethal effects of LPS by accelerating its clearance from the plasma, redirecting it away from monocytes and macrophages, decreasing immune cell activation, and reducing the release of cytokines, thus attenuating LPS toxicity.

Although it is now established that lipoproteins can bind and inactivate LPS, the nature of this interaction is not completely understood. Furthermore, conflicting evidence exists regarding the necessary component(s) of lipoproteins (lipid vs. protein) that attenuates the toxic effects of LPS. Lipid emulsions, which are devoid of proteins, demonstrate LPS-neutralizing effects similar to those of TG-rich lipoproteins, suggesting that the protein component of the lipoproteins may not be necessary (295, 312, 313). Ultrastructural studies of the LPS-LDL complex also show that the fatty acyl chain of the toxic lipid A moiety of LPS is inserted into the phospholipid surface of lipoproteins, thus masking the active site of LPS (329). Furthermore, the phospholipid content, but not cholesterol, TG, or protein, correlates with the ability of lipoproteins to neutralize LPS (300). Recently, LPC, an endogenous phospholipid, was shown to protect mice from experimental sepsis (330).

On the other hand, certain proteins associated with lipoproteins can bind and help modulate the inactivation of LPS by lipoproteins. These proteins include LBP, PLTP, apoA-I, apoE, and apoA-IV.

LBP is a positive acute-phase protein carried on lipoproteins (331). During infection, the concentration of LBP in the circulation increases many-fold. LBP is associated with HDL, VLDL, LDL, and chylomicrons (332–334). LBP binds lipid A of LPS, modulating its effect. At low concentrations, LBP catalyzes the transfer of LPS to CD14 on the surface of monocytes and macrophages, resulting in cellular activation and enhancement of the effects of LPS. At higher concentrations, however, LBP transfers LPS to lipoproteins, where neutralization occurs (333). LBP is also produced in the intestine and in the lung, where it may play a role in local responses to bacterial LPS (335, 336). LBP-deficient mice are more susceptible to gram-negative bacterial infection (337), whereas systemic injection of LBP into animals treated with LPS or infected with bacteria reduces cytokine release and decreases mortality (338). PLTP, another HDL-associated protein, can also bind and transfer LPS to HDL (339). However, the role of PLTP in neutralizing the effects of LPS in intact animals is not known.

ApoA-I or apoA-IV alone decreases the activation of macrophages by LPS (327, 340). LPS preincubated with apoA-I in vitro reduces the febrile response in animals (298). Transgenic mice overexpressing apoA-I are resistant to LPS-induced lethality and severe gram-negative infections (315). Secretion of cytokines from lymphocytes of apoA-IV transgenic mice was less pronounced than that of control animals (340). Similarly, injection of apoE reduces the production of cytokines and death induced by

LPS (341). Although apoE-deficient mice have high levels of cholesterol, they are more susceptible to endotoxemia and gram-negative infections (342). The facts that high levels of cholesterol could not protect apoE-deficient mice from the toxic effects of LPS and that these mice develop defects in the phagocytic activity of granulocytes suggest that apoE may have additional effects on the immune system (343). It is of interest that macrophages themselves make and secrete apoE (344). ApoA-IV was recently found to be increased in HDL during the APR (our unpublished observations).

Thus, more than one component of lipoproteins may induce the binding and inactivation of LPS. The interaction between LPS and lipoproteins may involve lipids, but proteins, such as LBP, may help catalyze the process. The metabolism of lipoprotein-bound LPS is altered such that it is shunted away from the activation of the monocytes/macrophages, ameliorating its toxic effect and accelerating clearance. The increases in TG-rich lipoproteins and LBP during sepsis may therefore be beneficial to the host during bacterial infection.

Besides LPS from gram-negative bacteria, lipoproteins also neutralize the toxic effects of LTA from gram-positive bacteria (301). Native lipoproteins or synthetic lipids inhibited the activation of macrophages by LTA. Similarly, this effect of lipoproteins on LTA requires LBP (301).

Lipoproteins, lipoprotein receptors, and viruses

Lipoproteins also bind and neutralize a wide variety of enveloped and nonenveloped DNA and RNA viruses. These include New Castle disease virus, Rabies virus, Vesicular stomatitis virus, Japanese encephalitis virus, Rubella virus, Epstein-Barr virus, Herpes simplex virus, HIV, Simian immunodeficiency virus, Xenotropic virus, Sindbis virus, Vaccinia virus, Coxsackie virus, Poliovirus, and Mengo virus (345–356). VLDL and LDL are particularly active against certain viruses, such as togaviruses (Japanese encephalitis virus and Rubella virus) and rhabdoviruses (Rabies virus and Vesicular stomatitis virus), whereas HDL displays a broader antiviral activity (347, 349, 356). However, it is estimated that HDL accounts for only a modest degree of total antiviral activity in serum (356).

When lipoproteins were separated into lipid and protein components, it was found that neutralization of some viruses was attributable to lipid moieties, especially phospholipid and cholesterol (351, 357–360). However, apolipoproteins also bind and inactivate viruses. Certain viruses possess envelope glycoproteins that contain amphipathic α-helix peptides. Because apoA-I and synthetic amphipathic peptide analogs inhibit virus-induced cell fusion (352), it has been proposed that the amphipathic peptides of apoA-I and other apolipoproteins may interfere with membrane fusion and entry of the virus into the host cell. Displacement of apoA-I on HDL by apoSAA during infection may provide free apoA-I for this purpose. When cells were infected with viruses in the presence of HDL, viruses were retained on the cell surface, suggesting that HDL inhibits viral penetration into cells (356).

Cellular GSLs are exploited as receptors by a number of microorganisms, including viruses and bacteria (361). Because acute-phase lipoproteins are enriched in GSLs (128), they may prevent the entry of these organisms.

Viral infection leads to the induction of IFNs, which in turn induce several antiviral proteins. One of these proteins is a soluble form of LDL receptor comprising the ligand binding domain, which displays antiviral activity by interfering with virus assembly or budding (362). A recombinant soluble LDL receptor fragment has been found to inhibit human rhinovirus infection (363). An increase in LDL in rodents during infection may help compete with viruses for cellular uptake, protecting the host against viral infection. Besides soluble LDL receptor, cells infected with virus also shed a VLDL receptor fragment that binds human rhinovirus, inhibiting viral infection of cells (364). Because viruses, such as rhinovirus and hepatitis C virus, use the LDL receptor for entry into cells (365, 366), the increases in VLDL in all animal species and increases in LDL in rodents may help compete with these or similar viruses for cellular uptake, protecting the host against viral infection.

Lipoproteins and parasites

Lipoproteins protect from certain parasitic infections. Trypanosomes are unicellular parasites that cause sleeping sickness in animals. Humans are susceptible to infection by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. However, the closely related subspecies *Trypanosoma brucei brucei* does not cause infection in humans because those trypanosomes are subject to lysis by human serum. Two distinct serum trypanosome lytic factors (TLFs), TLF1 and TLF2, have been characterized (367). TLF1 is a subset of lipid-rich HDL that contains mostly apoA-I and haptoglobin-related protein with trace amounts of apoA-II, haptoglobin, and PON. TLF2, in contrast, is a lipid-poor lipoprotein complex composed of apoA-I, haptoglobin-related protein, and immunoglobulin M. TLF2 accounts for most of the TLF activity in serum, as physiological levels of haptoglobin present in serum inhibit endogenous TLF1 activity (367). The mechanism of trypanolysis by TLFs is currently not known; evidence does not support the hypothesis that peroxidation is involved (368). Recent work implicates apoL-I, another HDL-associated protein, as a TLF in serum (369). ApoL-I interacts with serum resistance-associated protein in the lysosome of trypanosomes. Depletion of apoL-I from normal serum abolished the trypanolytic activity, whereas addition of native or recombinant apoL-I restored the activity (369).

Schistosomiasis is a parasitic infection of the hepatic portal system caused by schistosomes. Resistance to *Schistosoma* infection may be mediated by lipoproteins through several mechanisms. In rats, *Schistosoma* infection causes an increase in serum levels of CRP, a positive acute-phase protein associated with VLDL and LDL. CRP has been shown to activate platelets and render them cytotoxic to schistosomula in vitro (370). Besides platelets, activated monocytes can kill schistosomula. Because LDL and oxi-

dized LDL bind to the surface of schistosomula, it is thought that activated monocytes generate toxic oxygen species, which oxidize parasite-bound LDL, allowing endocytosis of the oxidized LDL into monocytes via the scavenger receptor (371). Removal of bound LDL exposes the parasites to further attack by activated monocytes and other immune cells.

Malaria infection is initiated after injection of malaria sporozoites into the bloodstream by mosquitoes. Hepatic invasion of malaria sporozoites is an initial step in the life cycle of the parasite. Malaria sporozoites and remnant lipoproteins of chylomicrons and VLDL are cleared from plasma using similar mechanisms (372). Malaria sporozoites are less infectious in LDL receptor-deficient mice maintained on a high-fat diet compared with those on a chow diet, suggesting that high levels of lipoproteins inhibit sporozoite infectivity in mice (372).

Oxidized phospholipids and LPS signaling

Infection and inflammation are associated with increased oxidized lipids (258). One of the oxidized phospholipids, oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (oxPAPC), inhibits LPS-stimulated NF-κB activation in monocytes/macrophages and endothelial cells by disrupting caveolae and inhibiting the assembly of the LPS signaling complex in lipid rafts (373). In addition, oxPAPC blocks the binding of LPS to LBP and CD14 (374). As a result, the LPS-induced expression of IL-8, IL-12, monocyte chemoattractant protein-1, and E-selectin is reduced. The ability of oxidized phospholipids to modulate the LPS signaling could be beneficial to the host during infection/inflammation. In fact, oxidized phospholipids have been shown to decrease an inflammatory process in mice treated with LPS, protecting them from endotoxic shock (374).

Lipoproteins and redistribution of lipids to immune cells

During infection/inflammation, there is an increase in TG-rich VLDL particles, which could provide lipid substrates for the activated immune system. In the presence of LPS, macrophages accumulated more TG and cholesterol (286, 287). VLDL produced during endotoxemia also provided more TG to macrophages compared with control VLDL, and these TGs were selectively stored as cellular lipids (375). During the APR, proteins involved in the uptake and metabolism of FA, such as FABP, FATP, and LPL, are coordinately downregulated in the heart, muscle, and adipose tissue. As a result, fat oxidation in the heart and skeletal muscle decreases, whereas adipose tissue does not store fat but rather provides FA for use by other tissues.

Similarly, during infection there is a decrease in HDL and the RCT pathway, which helps conserve cholesterol at peripheral sites. An increase in apoSAA on acute-phase HDL helps redirect cholesterol away from catabolism by hepatocytes and delivers cholesterol to other cells, such as macrophages (376). Upregulation of $sPLA_2$ increases cholesteryl ester uptake into the adrenal glands during the APR, presumably for increased steroid hormone synthesis (377). Cholesterol may also be used for lymphocyte activation and proliferation (378). Furthermore, infection is often associated with cellular injury, and areas of injury may need extra cholesterol for new membrane synthesis.

CONCLUSION

Infection and inflammation are associated with marked changes in lipid and lipoprotein metabolism. Besides their role in lipid transport, lipoproteins participate in innate immunity, which is the first line of host defense against invading microorganisms. Many of the changes in lipoproteins during infection/inflammation help protect the host from harmful effects of the stimuli. In cases of chronic infection, inflammatory diseases, diabetes, obesity, metabolic syndrome, and heart failure, however, these cytokine-induced changes in the structure and function of lipoproteins could be deleterious and may contribute to the development of atherosclerosis. Further studies of the interface between infection/inflammation and lipoproteins could provide new insights into not only atherogenesis but also the innate immune system and the complex interaction between them.

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