

- [24] Lazzeri G, Rossi S, Pammolli A, Pilato V, Pozzi T, Giacchi MV. Underweight and overweight among children and adolescents in Tuscany (Italy). Prevalence and short-term trends. *J Prev Med Hyg* 2008;49:13–21.
- [25] Pinhas-Hamiel O, Zeitler P. The global spread of type 2 diabetes mellitus in children and adolescents. *J Pediatr* 2005;146:693–700.
- [26] Nadeau KJ, Zeitler PS, Bauer TA, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. *J Clin Endocrinol Metab* October 2009;94:3687–95.
- [27] Eagle TF, Gurm R, Goldberg CS, et al. Health status and behavior among middle-school children in a midwest community: what are the underpinnings of childhood obesity? *Am Heart J* 2010;160:1185–9.
- [28] Kannel WB, Kannel C, Paffenbarger Jr RS, Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. *Am Heart J* 1987;113:1489–94.
- [29] Kwok SY, So HK, Choi KC, et al. Resting heart rate in children and adolescents: association with blood pressure, exercise and obesity. *Arch Dis Child* 2013;98:287–91.
- [30] Wändell PE, Carlsson AC, Theobald H. The association between BMI value and long-term mortality. *Int J Obes* 2009;33:577–82.
- [31] Rennie KL, Livingstone MBE, Wells JCK, et al. Association of physical activity with body-composition indexes in children aged 6–8 y at varied risk of obesity. *Am J Clin Nutr* 2005;82:13–20.
- [32] Török K, Szelényi Z, Pórszász J, Molnár D. Low physical performance in obese adolescent boys with metabolic syndrome. *Int J Obes Relat Metab Disord* 2001;25:966–70.
- [33] Gondoni LA, Titon AM, Nibbio F, Augello G, Caetani G, Liuzzi A. Heart rate behavior during an exercise stress test in obese patients. *Nutr Metab Cardiovasc Dis* 2009;19:170–6.
- [34] Gutin B, Islam S, Treiber F, Smith C, Manos T. Fasting insulin concentration is related to cardiovascular reactivity to exercise in children. *Pediatrics* 1995;96:1123–5.
- [35] Laguna M, Aznar S, Lara MT, Lucía A, Ruiz JR. Heart rate recovery is associated with obesity traits and related cardiometabolic risk factors in children and adolescents. *Nutr Metab Cardiovasc Dis* Dec 1 2012, <http://dx.doi.org/10.1016/j.numecd.2012.10.002>.
- [36] Lin LY, Kuo HK, Lai LP, Lin JL, Tseng CD, Hwang JJ. Inverse correlation between heart rate recovery and metabolic risks in healthy children and adolescents: insight from the National Health and Nutrition Examination Survey 1999–2002. *Diabetes Care* 2008;31:1015–20.
- [37] Cheng YJ, Macera CA, Church TS, Blair SN. Heart rate reserve as a predictor of cardiovascular and all-cause mortality in men. *Med Sci Sports Exerc* 2002;34:1873–8.
- [38] Wändell PE, Carlsson AC, Theobald H. Effect of heart-rate recovery on long-term mortality among men and women. *Int J Cardiol* 2010;144:276–9.
- [39] Cheng YJ, Lauer MS, Earnest CP, et al. Heart rate recovery following maximal exercise testing as a predictor of cardiovascular disease and all-cause mortality in men with diabetes. *Diabetes Care* 2003;26:2052–7.
- [40] Deniz F, Katircibasi MT, Pamukcu B, Binici S, Sanisoglu SY. Association of metabolic syndrome with impaired heart rate recovery and low exercise capacity in young male adults. *Clin Endocrinol* 2007;66:218–23.
- [41] Brinkworth GD, Noakes M, Buckley JD, Clifton PM. Weight loss improves heart rate recovery in overweight and obese men with features of the metabolic syndrome. *Am Heart J* 2006;152:693.e1–6.
- [42] Owens S, Gutin B, Allison J, et al. Effect of physical training on total and visceral fat in obese children. *Med Sci Sports Exerc* 1999;31:143–8.
- [43] Bell LM, Watts K, Siafarikas A, et al. Exercise alone reduces insulin resistance in obese children independently of changes in body composition. *J Clin Endocrinol Metab* 2007;92:4230–5.
- [44] Brunetti ND, Amoroso D, De Gennaro L, et al. Tele-medicine for cardiovascular emergencies: ready for the prime time? *J Clin Exp Cardiol* 2012;3:8.

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Intravenous administration of Gr-1+CD11b+ myeloid cells increases neovascularization and improves cardiac function after heart infarction

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The relative importance of bone marrow-derived cell populations to adult neovascularization is not clear. There are increasing evidences that myeloid cell lineage may play a role in neovascularization [1–4]. In a previous report, we demonstrated that Gr-1+CD11b+ myeloid cells improve both angiogenesis and vasculogenesis in tumor [1]. A recent report by Kim et al. showed that muscle-derived Gr1(dim)CD11b(+) myeloid cells enhance neovascularization in an ischemic hind limb

model [3]. Similarly, a study with the same ischemic hindlimb model also verified the enhanced neovascularization properties uniquely associated with proangiogenic cells derived from common myeloid progenitors [4]. In the present study, we further investigated whether intravenous administration of Gr1+CD11b+ myeloid cells increases neovascularization and improves cardiac function after heart infarction.

C57BL/6 mice were purchased from Jackson Lab. All animals received humane care and the study protocols were approved by the Vanderbilt Medical University Animal Care and Use Committee.

We sorted Gr-1+CD11b+ myeloid cells from the spleens of mice as previously described [5]. To investigate whether Gr-1+CD11b+ myeloid cells increase angiogenesis *in vitro*, we cultured Gr-1+CD11b+ myeloid cells with aortic ring [6]. At the seventh day, we found that endothelial sprouts in Gr-1+CD11b+ myeloid cell treated group are significantly more than that in no cell treated group (23.2 ± 3.7 versus 13.4 ± 2.4 control, $p < 0.01$, $n = 5$ in each group, Fig. 1A and B), which indicated that Gr-1+CD11b+ myeloid cells increase angiogenesis *in vitro*. In order to see whether Gr-1+CD11b+ myeloid cells increase angiogenesis *in vivo*, we performed dorsal window model in mice [7]. We injected Gr-1+CD11b+ myeloid cells into tail vein of mice to see whether injected Gr-1+CD11b+ myeloid cells home to the site of window chamber. By tracing Gr-1+CD11b+ myeloid cells marked with PKH-26 (Fig. 1C-a), we found that the Gr-1+CD11b+ myeloid cells home to the site of the window chamber after intravenous injection (Fig. 1C-b). We further found the intravenous injection of Gr-1+CD11b+ myeloid cells that obviously increase blood vessel branches

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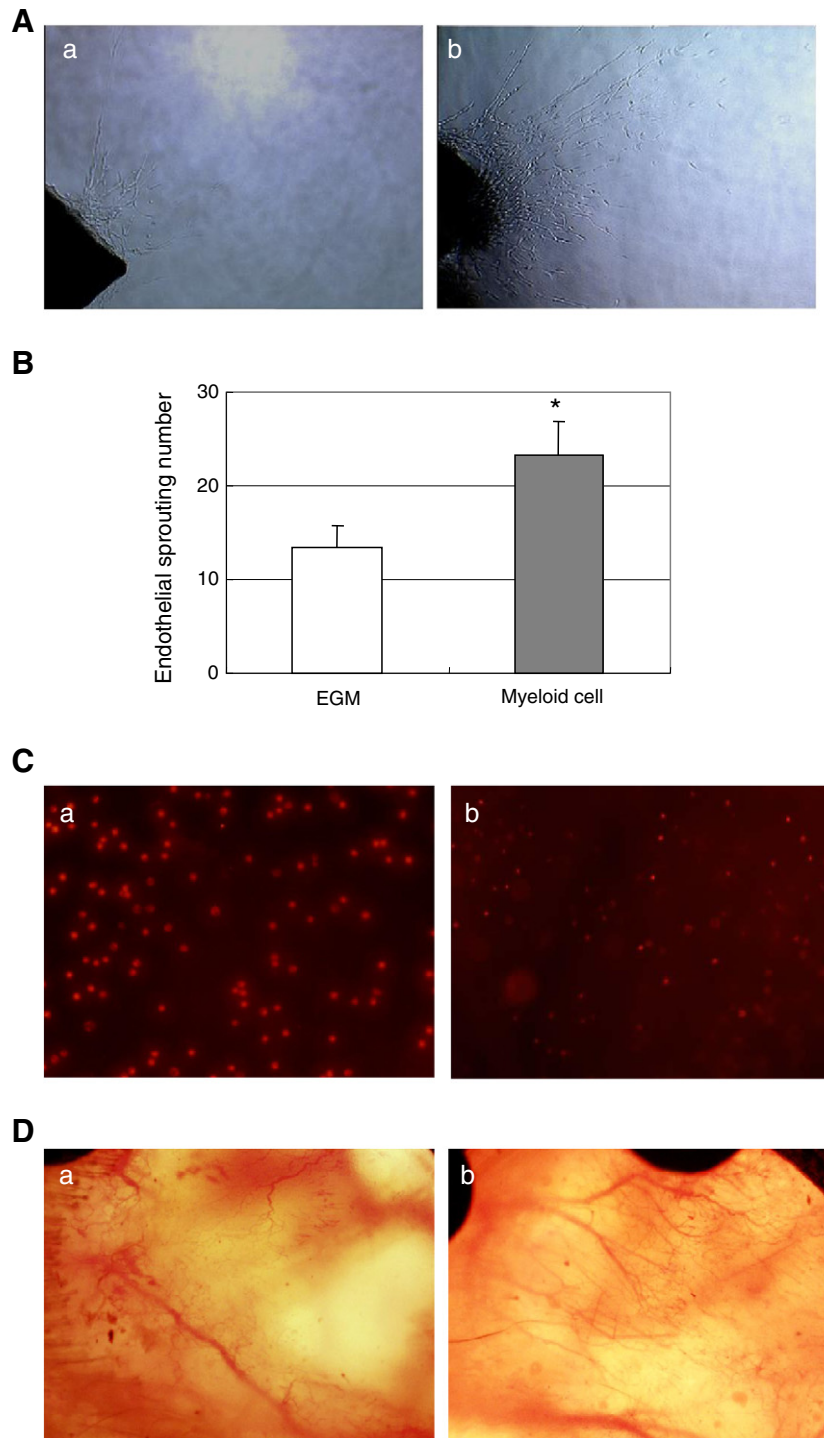


Fig. 1. Gr-1+CD11b+ myeloid cells increase angiogenesis *in vitro* and *in vivo*. (A) Representative endothelial sprouts after 7 days of aortic ring sprouts assay. a, aorta ring culture with no cells; b, aorta ring culture with Gr-1+CD11b+ myeloid cells. Magnification $\times 10$. (B) Gr-1+CD11b+ myeloid cells significantly increase endothelial sprouts compared with no cell treatment, $*p < 0.01$ versus EGM. (C) Gr-1+CD11b+ myeloid cells home to the site of window chamber after injection from tail vein. a, Gr-1+CD11b+ myeloid cells marked with PKH-26. Magnification $\times 100$. b, Gr-1+CD11b+ myeloid cells homed to the site of window chamber. Magnification $\times 40$. (D) Gr-1+CD11b+ myeloid cells increase angiogenesis in window chamber. a, saline treatment; b, Gr-1+CD11b+ myeloid cell treatment. Magnification $\times 10$.

at the site of window chamber, compared with saline treated groups, which suggested that the Gr-1+CD11b+ myeloid cells also increase angiogenesis *in vivo* (Fig. 1D).

Next, we injected Gr-1+CD11b+ myeloid cells *via* tail vein to see whether Gr-1+CD11b+ myeloid cells improve heart function after heart infarction with a model of LAD ligation in mice [8]. After 4 weeks of LAD ligation and myeloid cell injection, the cardiac function was evaluated by echocardiography. The measured index indicated that

myeloid cells significantly improve heart FS ($37.2 \pm 7.8\%$ versus $27.3 \pm 7.2\%$ control, $p < 0.05$, $n = 5$ in each group, Fig. 2A and B). The hearts were harvested after 4 weeks, and Masson's trichrome staining was performed [9]. The results indicated that myeloid cells significantly decrease infarct size after LAD ligation ($28.9 \pm 7.4\%$ versus $45.8 \pm 5.0\%$ control, $p < 0.01$, $n = 5$ in each group, Fig. 2C and D). We further performed capillary density measurement and tracing myeloid cells marked with PKH-26 in the infarct hearts. The results showed that

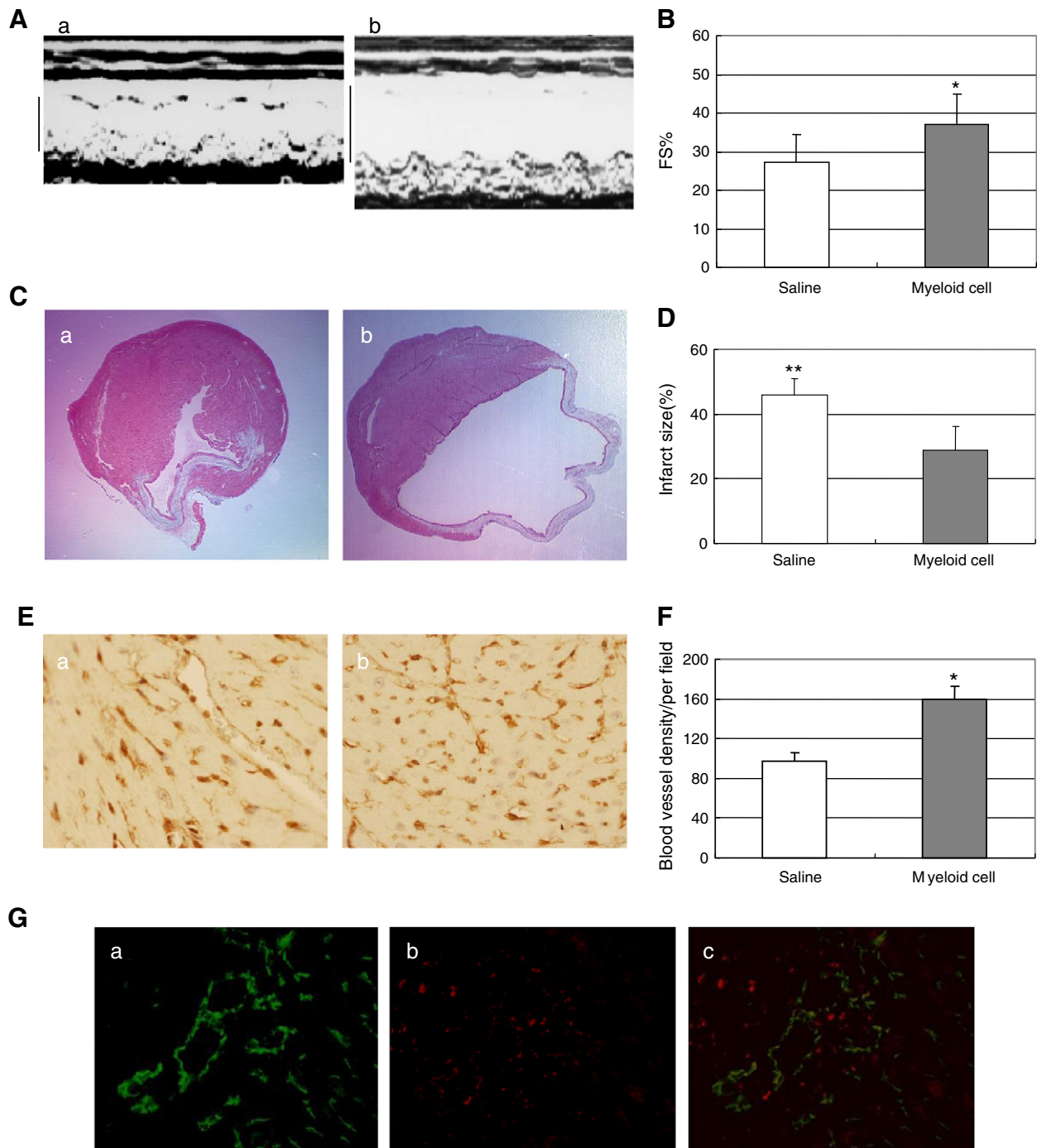


Fig. 2. Intravenous injection of Gr-1+CD11b+ myeloid cells improves cardiac function and decreases cardiac infarct size after heart infarction *via* increasing neovascularization. (A) Representative echocardiography of heart after 4 weeks of LAD ligation. a, Gr-1+CD11b+ myeloid cells injected from tail vein; b, saline injected from tail vein. (B) Gr-1+CD11b+ myeloid cells significantly improve FS after heart infarction compared with saline, * $p < 0.05$ versus saline, $n = 5$ in each group. (C) Representative Masson's trichrome staining of the heart after 4 weeks of heart infarction. a, Gr-1+CD11b+ myeloid cell treatment; b, saline treatment. (D) Gr-1+CD11b+ myeloid cells significantly decrease cardiac infarct size after heart infarction compared with saline, ** $p < 0.01$ versus saline, $n = 5$ in each group. (E) Representative capillary density measurement in peri-infarct area of the heart. a, saline treatment; b, Gr-1+CD11b+ myeloid cell treatment. Magnification $\times 200$. (F) Gr-1+CD11b+ myeloid cells significantly increase angiogenesis in the peri-infarct area after heart infarction, * $p < 0.01$ versus saline, $n = 5$ in each group. (G) Florescent imaging of the heart after intravenous injection of Gr-1+CD11b+ myeloid cells. a, endothelial cells stained with anti-CD31 (green); b, Gr-1+CD11b+ myeloid cells marked with PKH-26 (red); c, some of the Gr-1+CD11b+ myeloid cells incorporate into vasculature (yellow). Magnification $\times 200$.

myeloid cells significantly increase capillary density in the board area of infarction (159.2 ± 13.3 versus 96.8 ± 9.0 control, $p < 0.01$, $n = 5$ in each group Fig. 2E and F). By tracing the myeloid cells and immunostaining of endothelial cells with anti-CD31 antibody, we found a few Gr-1+CD11b+ myeloid cells incorporate into vasculature

(Fig. 2G). These indicated that Gr-1+CD11b+ myeloid cells improve cardiac function after heart infarction *via* both angiogenesis and vasculogenesis.

In conclusion, the intravenous administration of Gr-1+CD11b+ myeloid cells increases neovascularization and improves cardiac

function after heart infarction. The Gr-1+CD11b+ myeloid cells could be used as a potential cell source in the field of cell therapy for ischemic cardiovascular diseases.

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References

- [1] Yang L, DeBusk LM, Fukuda K, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 2004;6:409–21.
- [2] Grunewald M, Avraham I, Dor Y, et al. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006;124:175–89.
- [3] Kim JA, March K, Chae HD, et al. Muscle-derived Gr1(dim)CD11b(+) cells enhance neovascularization in an ischemic hind limb mouse model. *Blood* 2010;116(9):1623–6.
- [4] Wara AK, Croce K, Foo S, et al. Bone marrow-derived CMPs and GMPs represent highly functional proangiogenic cells: implications for ischemic cardiovascular disease. *Blood* 2011;118(24):6461–4.
- [5] Ljung BM, Mayall B, Lottich C, et al. Cell dissociation techniques in human breast cancer—variations in tumor cell viability and DNA ploidy. *Breast Cancer Res Treat* 1989;13:153–9.
- [6] Huang L, Sankar S, Lin C, et al. HCP1PA, a protein tyrosine phosphatase that regulates vascular endothelial growth factor receptor-mediated signal transduction and biological activity. *J Biol Chem* 1999;274:38183–8.
- [7] Lin P, Polverini P, Dewhirst M, Shan S, Rao PS, Peters K. Inhibition of tumor angiogenesis using a soluble receptor establishes a role for Tie2 in pathologic vascular growth. *J Clin Invest* 1997;100:2072–8.
- [8] Zhang R, Khoo MS, Wu Y, et al. Calmodulin kinase II inhibition protects against structural heart disease. *Nat Med* 2005;11:409–17.
- [9] Takahashi K, Ito Y, Morikawa M, et al. Adenoviral-delivered angiopoietin-1 reduces the infarction and attenuates the progression of cardiac dysfunction in the rat model of acute myocardial infarction. *Mol Ther* 2003;8:584–92.

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Serum IgE antibody concentrations and the risk of Kounis syndrome

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It is known that immunoglobulin E (IgE) is an important regulator of allergic reactions because it activates mast cells to release their inflammatory mediators by binding to high affinity FcεRI receptors situated on the mast cell surface. Inflammatory mediators such as histamine, neutral proteases, arachidonic acid products, platelet activating factor and a variety of cytokines and chemokines released during the activation process can induce coronary artery spasm and atheromatous plaque erosion and rupture culminating in the development of Kounis syndrome [1]. In addition to mast cells, other inflammatory cells such as dendritic cells, eosinophils, platelets, monocytes, and macrophages also bear high affinity receptors on their surfaces [2]. Therefore, IgE's targets likely extend beyond mast cells in order to induce coronary events and Kounis syndrome.

In the very interesting analysis published in this journal [3] Dr Ivy Shiue commenting on a national population-based multi-year cross-sectional study conducted by United States National Health and Nutritional Survey 2005–2006 (NHANES), wondered if higher serum IgE antibody concentrations are associated with adult cardiovascular disease. NHANES 2005–2006 collected questionnaire and laboratory data on United States citizens and was also the first nationwide

assessment of serum total IgE and specific IgE. This study showed that people with higher total IgE levels were likely to have coronary heart disease and only shrimp serum specific IgE antibody was significantly associated with coronary heart disease. Dr Shiue added that although an association between total serum IgE concentrations, shrimp specific antibody and coronary heart disease was found, causality cannot be established and suggested future studies with very large study populations.

Similar results have been found in previous studies. In a cross-sectional population study [4] which was carried out on a group of 262 men and 315 women, a statistically significant positive correlation between increased IgE levels in men and previous myocardial infarction, stroke and peripheral arterial disease was found irrespective of total cholesterol and LDL levels. In another study [5] concerning 156 patients with coronary heart disease who were admitted to cardiological wards, total IgE levels were found to be significantly higher in patients with unstable angina and acute myocardial infarction compared to patients with stable angina and controls. In a prospective [6] study of 621 patients followed for about nine years, a significant correlation between the increased IgE levels and the incidence of acute myocardial infarction in males was found. When IgE levels were above 200 kU/L the incidence of non-fatal myocardial infarction was seven times higher. In a recent study [7] of 195 patients suffering from ischemic heart disease serum IgE levels were increased as compared to the control group of healthy individuals. IgE serum levels on the days 1, 7, 14, and 40 after acute myocardial infarction did not revealed any significant changes. Males had significantly higher IgE than females.

However, the questions which arise are: first, increased IgE levels in acute coronary events are the result or precede coronary events. Second, elevated IgE levels are indicative of their participation in the development of the coronary event or are increasing due to inflammatory reaction from tissue damage. There are two studies answering to the above questions. In a prospective study, with initially healthy hyperlipidemic men who suffered myocardial infarction, the levels of IgE as well as of other immunoglobulin classes such as IgA, IgG were significantly higher than the matched healthy controls [8]. The risk of myocardial infarction increased with the increase of

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