The effect on mouse immune systems of cow's colostrum produced 6 to 7 days after parturition

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Five-week-old male mice were divided into 2 groups in which they were orally given sterile saline solution (control solution) or cow's colostrum powder prepared from milk produced 6 to 7 days after parturition in sterile saline solution (colostrum solution). The mice were given the solution once a day for 5 weeks. The level of intestinal total IgG was significantly lower in the mice given the colostrum solution than in the mice given the control solution, and the intestinal IgA and serum IgG levels tended to be lower in the mice given the colostrum solution. The numbers of spleen $CD11b^+$, $CD19^+$, and IFN-γ^+CD4^+ cells were also significantly lower in mice given the colostrum solution. DNA microarray analysis of mRNAs extracted from Peyer's patch cells showed that the gene expression of proteins relating to T cell activation of acquired immune responses or Fcε-mediated mast cell activation was obviously lower in the mice given the colostrum solution than in the mice given the control solution, whereas that of proteins relating to T regulatory cells or intestinal innate immune system was noticeably higher in mice given the colostrum solution. These results suggest that the oral ingestion of cow's colostrum suppresses the acquired immune system and type I allergic reactions, and enhances the innate immune system.

Zum Einfluss von Kuh-Kolostralmilch, gewonnen an den Tagen 6 und 7 nach dem Abkalben, auf das Immun system der Maus

Fünf Wochen alte männliche Mäuse wurden in zwei Gruppen unterteilt, die entweder sterile Kochsalzlösung (Kontroll-Lösung) oder Kolostralmilchpulver von Kühen, gewonnen 6-7 Tage nach dem Abkalben und aufgelöst in steriler Kochsalzlösung (Kolostralmilch-Lösung) erhielten. Die Lösungen wurden den Mäusen einmal pro Tag über einen Zeitraum von 5 Wochen verabreicht. Die Level an intestinalem Gesamt-IgG lagen bei den Mäusen, die die Kolostralmilch-Lösung erhalten hatten, signifikant niedriger im Vergleich zu den Mäusen mit Gabe der Kontroll-Lösung. Auch die intestinalen IgA- und Serum-IgG-Werte zeigten eine Tendenz zu niedrigeren Werten bei den Mäusen mit Gabe der Kolostralmilch-Lösung. Die Zahlen der in der Milz vorhandenen $CD11b^+$-, $CD19^+$- und IFN-γ^+CD4^+-Zellen waren ebenfalls signifikant niedriger bei Mäusen mit Gabe der Kolostralmilch-Lösung. Die DNA-Mikroarray-Analyse der mRNAs, extrahiert aus den Zellen der Peyer'schen Platten, zeigte, dass die Genexprimierung der Proteine in Bezug auf die T-Zellaktivierung der erworbenen Immunreaktion oder die Fcε vermittelte Mastzellen-Aktivierung deutlich niedriger waren bei Mäusen mit Gabe der Kolostralmilch-Lösung im Vergleich zu den Kontrolltieren. Demgegenüber war die Exprimierung der Proteine in Bezug auf die T-regulatorischen Zellen oder das intestinale innate (angeborene) Immunsystem deutlich höher bei Mäusen mit Verabreichung der Kolostralmilch-Lösung. Diese Ergebnisse weisen darauf hin, dass die orale Aufnahme von Kolostralmilch von Kühen das erworbene Immunsystem und damit allergische Reaktionen vom Typ I unterdrückt und das angeborene Immunsystem stärkt.

14 Colostrum (mouse immune system) **14 Kolostralmilch** (Immunsystem der Maus)

1. Introduction

The colostrum is the early milk produced during the first several days after parturition and contains a large amount of anti-infectious proteins such as IgG, secretory IgA, lactoferrin, and lysozyme, since mammalian neonates are immunologically immature.

On the other hand, some milk proteins and their digests have been demonstrated to modulate the development of immune systems. WONG and WATSON (1) demonstrated that the oral ingestion of cow's whey proteins had an enhancing effect on the secondary humoral antibody response in mice. OTANI *et al.* (2, 3) found that dietary cow's casein phosphopeptides (CPP) enhanced the intestinal IgA response to peritoneally or orally ingested antigens in piglets and mice. KITAMURA *et al.* (4) observed that the oral ingestion of CPP by pregnant sows produced higher levels of colostral IgA and IgG than in sows that did not ingest CPP during pregnancy. OHNUKI *et al.* (5) reported that the oral ingestion of cow's milk IgG suppressed immunoglobulin production in mice.

The concept of protecting a host with cow's colostrum is not new. The oral ingestion of the IgG-rich fraction of the colostrum of cows that had been immunized with infectious microorganisms has been demonstrated to provide effective protection against microorganism infections in some domesticated animals (6). Recently, YOSHIOKA *et al.* (7) observed that the colostrum produced during the first 4 d of post-parturition directly stimulated intestinal intraepithelial lymphocytes to develop into T helper 1 type (Th1), which might protect animals from infectious diseases and allergic diseases mediated via T helper 2 type (Th2) responses. These facts suggest that the colostrum produced during the early days of post-parturition is useful as an immunomodulatory supplement for infant formula and other physiologically functional foods. However, the colostrum produced during the first 5 d of post-parturition (p.p.) is legally restricted with regard to its use as a food and food supplement in Japan.

The aim of this study is to learn the immune properties of mice given cow's colostrum produced 6 to 7 days p.p.

2. Materials and methods

This experiment was conducted in accordance with the guidelines for the Regulation of Animal Experimentation at Shinshu University and according to Law No. 105 and Notification No. 6 of the Japanese government.

2.1 Materials

Defined fetal bovine serum (FBS) was obtained from Equitech-Bio Inc. (Kerrville, USA). RPMI-1640 medium was purchased from Nissui Pharmaceutical (Tokyo, Japan). Biolegend (San Diego, USA) was supplied phycoerythrin (PE)-labeled anti-mouse interleukin (IL)-4 monoclonal antibodies (mAb, clone 11B11), PE-labeled anti-mouse interferon (IFN)-γ mAb (clone XMG1.2), biotin-labeled anti-mouse CD4 mAb (clone RM4-5), biotin-labeled anti-mouse CD11b mAb (clone M1/70), biotin-labeled anti-mouse CD19b mAb (clone MB19-1), and phycoerythrin/cyanine 5 (PE/Cy5)-labeled streptavidin. Brefeldin A (BFA), ionomycin, and phorbol 12-myristate 13-acetate (PMA) were obtained from Wako Pure Chemical Industries (Osaka, Japan). IntraPrep Permeabilization Reagent was bought from Beckman Coulter Inc.m Tokyo. Penicillin G potassium and streptomycin sulfate were obtained from Meiji Seika, Tokyo. Guava Viacount Reagent was purchased from Guava Technologies (Hayward, USA). Other chemicals were of the highest analytical grade commercially available.

2.2 Colostrum powder

Colostrum powder was prepared from cow's colostrum that was produced 6 to 7 d after parturition. Briefly, the colostrum was centrifuged to partially remove the fat, filtered with a membrane to partially remove low molecular substances, heat-treated at 73°C for 15 sec, and spray-dried. The colostrum powder consisted of 49.8% protein including 11.0% IgG, 36.4% lactose, 1.8% fat, 7.8% mineral, and 4.2% moisture.

2.3 Oral administration

Four-week-old male C3H/HeN mice were obtained from Japan SLC (Shizuoka, Japan). After preliminary breeding for 1 week, the mice were divided into 2 groups in which they were orally given 0.5 ml of sterile saline solution (control solution) or 8 mg of the colostrum powder dissolved in sterile saline solution with a total volume of 0.5 ml (colostrum solution). Each group consisted of 7 mice, and the mice were given the solution once a day for 5 weeks. Commercial mouse pellets (MF, Oriental Yeast Co., Tokyo) were continuously available from stainless-steel feeders, and water was provided ad libitum from drinking bottles. The mice were maintained at 24 ± 2°C under 12-h light/12-h dark cycles. The blood, spleen, intestinal tract, and Peyer's patch were collected from the mice after breeding them for 5 weeks.

2.4 Preparation of serum and intestinal extract

Serum was prepared from blood. One gram of the intestinal tract (duodenum to rectum) consisting of its tissues and contents was ground for 20 min at 2 ± 1 °C with sea sand (1 g) in 0.01 M sodium phosphate buffer at pH 7.2, containing 0.15 M sodium chloride (2.5 ml). The ground material was centrifuged at 1,200 g for 30 min at 4 °C, and the supernatant was collected as intestinal extract.

2.5 Preparation of spleen and Peyer's patch cells

Spleen and Peyer's patch cells were prepared according to the procedure described in a previous paper (8).

2.6 Immunoglobulin assay

The level of total IgG and IgA in the serum and intestinal extracts was measured according to the manufacturer's protocol with a Mouse IgG ELISA Quantitation Kit or a Mouse IgA ELISA Quantitation Kit (Bethyl Laboratories, Inc, Montgomery, USA), respectively.

2.7 Cell function analysis

The spleen cells were incubated with mouse Fc block for 15 min at 4℃ before being reacted with biotin-labeled anti-mouse mAbs specific for CD11b or CD19 for 15 min at 4℃. Cells that had already been reacted with the biotin-labeled antibody were further incubated with PE/Cy5-labeled streptavidin for 15 min at 4℃. Finally, all cells were analyzed using a Guava Personal Cell Function Analyzer (Guava PCA, Guava Technologies). For the analysis of $CD4^+$cell cytokines, spleen cells were incubated at 37 ℃ in RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 20 µg/ml BFA, 2 µg/ml ionomycin, and 20 ng/ml PMA for 4 h. The surface marker antigens of the cells were reacted with biotin-labeled anti-mouse mAbs specific for CD4 (clone RM4-5) for 15 min at 4 ℃ and were then incubated with PE/Cy5-labeled streptavidin for 15 min at 4 ℃. Intracellular cytokines were measured by permeabilizing cells before incubating them with PE-labeled anti-mouse cytokine mAbs specific for IL-4 or IFN-γ. Briefly, the cells were fixed in IntraPrep regent 1, and then after 15 min, the cells were washed and permeabilized by incubation with IntraPrep regent 2. The cells that contained cytokines were visualized after incubation with PE-labeled anti-mouse mAbs specific for IL-4 or IFN-γ and were analyzed by means of Guava PCA.

2.8 DNA microarray analysis

The genome-wide gene expression of Peyer's patch cells was examined according to the procedure described previously (9) by using the Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, USA). Data are shown as relative expression levels calculated as follows: Fluorescence intensity based on the mRNAs extracted from mice given the colostrum solution/fluorescence intensity based on the mRNAs extracted from mice given the control solution.

2.9 Statistical analysis

Data were expressed as mean ± standard deviation. The significance of differences was tested with the Student's *t*-test.

3. Results and discussion

3.1 Immunoglobulin levels in serum and intestinal tracts

The mice that were given the colostrum solution

gained body weight in a quite similar manner to those given the control solution (data not shown). Hence, there is little difference in the nutritional value of the control and colostrum solutions.

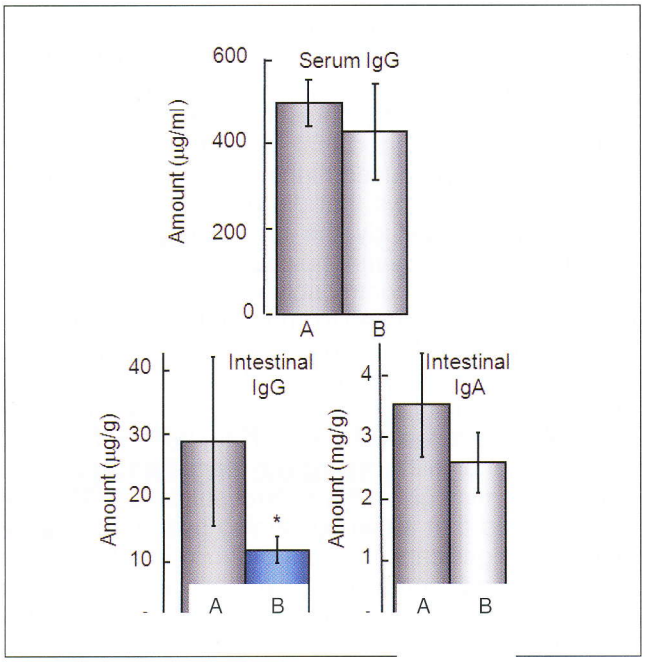

Fig. 1: Immunoglobulin levels in seru stinal tracts of mice given the control (A) or solution (B). *P<0.05

Figure 1 shows the levels of ; intestinal immunoglobulins. The intestinal l was significantly lower in the mice given th m solution than in the mice given the control The serum IgG and intestinal IgA levels also t e lower in the mice given the colostrum solut results indicate that the oral ingestion of colostrum suppresses immunoglobulin production.

3.3 Number of immunocompetent cells in spleens

Figure 2 shows the numbers of spleen $CD11b^+$, $CD19^+$, $IFN\text{-}\gamma^+CD4^+$, and $IL\text{-}4^+CD4^+$ cells. The numbers of $CD11b^+$, $CD19^+$, and $IFN\text{-}\gamma^+CD4^+$ cells were significantly lower in the mice given the colostrum solution than in the mice given the control solution. CD11b and CD19 are typical cell surface antigens of antigen-presenting cells and B cells, respectively (10, 11). $IFN\gamma^+CD4^+$ and $IL\text{-}4^+CD4^+$ cells are Th1 and Th2 cells, respectively, and stimulate the cellular immune system and the humoral immune system, respectively (12). These results suggest that the suppression of immunoglobulin production caused by the ingestion of colostrum might be attributable to the reduction of $CD11b^+$ and/or $CD19^+$ cell numbers.

3.4 Gene expression of immune system proteins in Peyer's patch cells

The mRNA extracted from Peyer's patch cells was subjected to DNA microarray analysis. Of about 39000 genes, the fluorescence intensity of 140 spots was at least 1.5-fold higher in the mice given the colostrum solution than in the mice given the control solution, while that of 32 spots was less than 0.5-fold lower in the former than in the latter. Out of the genes that showed a

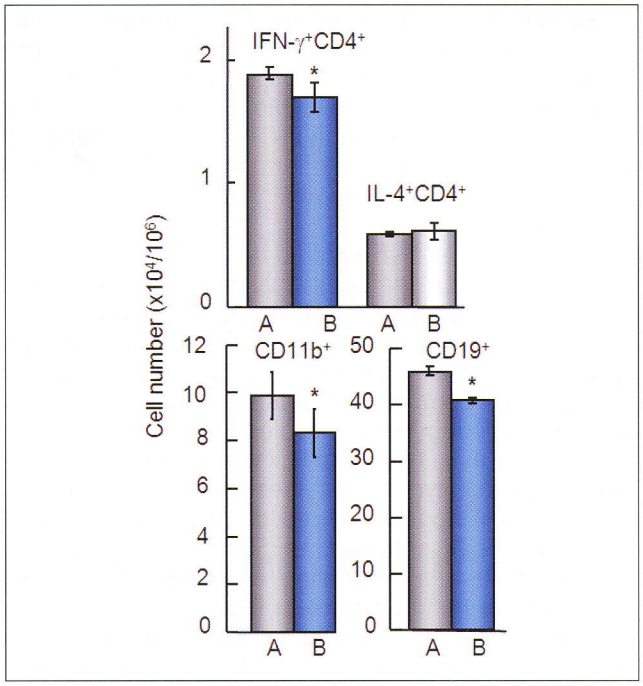

Fig. 2: Numbers of immunocompetent cells in spleens of mice given the control (A) or colostrum solution (B). *P<0.05

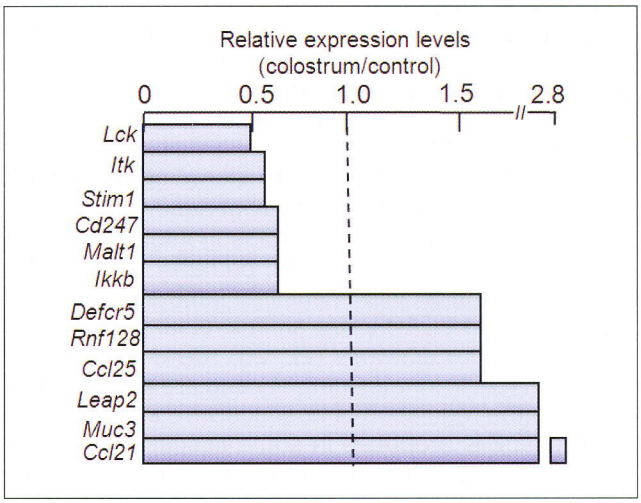

Fig. 3: Gene expression of proteins relating to immune systems in Peyer's patch cells of mice given the control or colostrum solution.

difference in expression between the ingestion of the control solution and the colostrum solution, the expression levels of those genes that relate to the immune system are shown in Fig. 3. The levels of *Lck*, *Itk*, *Stim1*, *Cd247*, *Malt1*, and *Ikkb* expression decreased to approximately half of their control values in the mice given the colostrum solution. In contrast, *Defcr5*, *Rnf128*, *Ccl25*, *Leap2*, *Muc3*, and *Ccl21* expression increased to more than 1.5-fold of the control values in the mice given the colostrum solution. The proteins transcribed from *Lck*, *Itk*, *Stim1*, *Cd247*, *Malt1*, and *Ikkb* are leukocyte-specific protein tyrosine kinase (Lck), IL-2-inducible T cell kinase (Itk), stromal interacting molecule 1 (STIM1), T cell receptor zeta (TCRzeta: CD247), mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1 (MALT1), and 3-phosphoinositide-

dependent protein kinase-1-mediated IkappaB kinase beta (IkkB), respectively. Similarly, the transcribed proteins from *Defcr5*, *Rnf128*, *Ccl25*, *Leap2*, *Muc3*, and *Ccl21* are defensin related cryptdin 5, ring finger protein 128 (GRAIL), chemokine (C-C motif) ligand 25 (CCL25), liver-expressed antimicrobial peptide 2 (Leap2), mucin 3, and chemokine (C-C motif) ligand 21 (CCL21), respectively.

The activation of NF-kappaB by T cell receptor (TCR) signaling is critical for T cell activation during the adaptive immune response. TCRzeta (CD247) functions as an amplification module in the TCR signaling cascade and is essential for assembly and surface expression of the TCR/CD3 complex (13). Malt1 controls T cell activation by regulating key molecules in TCR-induced signaling pathways (14), while Lck is a member of the Src family of protein tyrosine kinases and is critical for the T cell activation triggered by receptor ligation (15). In addition, IkkB is essential for rapid NF-kappa B activation by proinflammatory signaling cascades (16). These facts and the results shown in Fig. 3 indicate that the oral ingestion of the colostrum solution suppresses the adaptive immune response. On the other hand, Itk is produced by T cells and mast cells, and the reduction of Itk production reduces allergen/IgE-induced histamine release (17). STIM1 is important for promoting Ca^{2+} influx, which is essential for FcεRI-mediated mast cell activation and anaphylaxis (18). GRAIL is expressed in $CD4^{+}CD25^{+}$ T regulatory cells, and is linked to their functional regulatory activity (19). These facts and the results shown in Fig. 3 indicate that the oral ingestion of the colostrum solution reduces type I allergic reactions. Moreover, CCL21 and CCL25 are small cytokines that belonging to the CC-chemokinekine family. CCL21 stimulates the phagocytic activity of dendritic cells (20), while CCL25 plays essential roles in intestinal homing of IgA antibody-secreting cells, primarily by mediating their extravasation into intenstinal lamina propria (21). Defensins and LEAP-2 are antimicrobial peptides with broad-spectrum activities (22, 23). Defensins play an important role in innate immunity and are known to contribute to the regulation of host adaptive immunity (22), while Leap2 is produced in some tissues including intestinal tissues and is known to function as the body's "natural antibiotics" (23). Mucin 3 is a heavily glycosylated protein that protects epithelial membranes and is known to act as an important intestinal barrier (24). These facts and the results shown in Fig. 3 indicate that the oral ingestion of the colostrum solution enhances mucosal immunity, in particular, innate intestinal immunity. Thus, the DNA microarray analysis data show that the colostum produced 6 to 7 days after parturition suppresses adaptive immune responses, reduces type I allergic reactions, and enhances innate immunity.

The colostrum powder used in this study consisted of 49.8% protein including 11.0% IgG, as described in the "Materials and methods" section. OHNUKI and OTANI (8) demonstrated that the complex of antigens and their specific milk IgG boud to accessory cells through FcγRIIb and suppressed immunoglobulin production, although the antigen-free milk IgG bound to accessory cells through the FcγRI and increased immunoglobulin production. SUEDA and OTANI (9) reported that a diet including antigens and their specific milk IgG suppressed the production of immunoglobulin and the development of allergic syndromes through several mechanisms. Thus, it is suggested that IgG in orally administered colostrum might form antigen-antibody complexes with intestinal bacteria, and act strongly on the intestinal immune system.

Casein was also present in the colostrum powder used in this study. Casein is digested in the gastrointestinal tract and produces CPP. OTANI *et al.* (2, 3) found that CPP enhanced the intestinal IgA response. KITAMURA *et al.* (4) reported that the ingestion of CPP by pregnant sows produced higher levels of colostral IgA and IgG than in sows that did not ingest CPP during pregnancy. In this study, the intestinal IgA level tended to be lower in mice given the colostrum solution than in the mice given the control solution, but the expression of *Ccl25*, which is related to important roles in the intestinal homing of IgA antibody-secreting cells (21), was higher in the mice given the colostrum solution. The high expression of *Ccl25* might be attributable to the caseins in the colostrum. In addition, the level of *Muc3* was also higher in the mice given the colostrum solution than in the mice given the control solution. HAN *et al.* (25) reported that a diet containing hydrolyzed casein induced a significant increase of *Muc3* in rats, in comparison to a diet containing a synthetic amino acid mixture. The higher expression of *Muc3* in the mice given the colostrum solution might be attributable to caseins.

In conclusion, the authors propose that the cow's colostrum produced 6 to 7 days after parturition would be useful as a mucosal immunomodulatory and/or anti-allergic food ingredients.

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Managing condensing temperature to improve the efficiency of refrigeration system

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The mechanical vapour recompression type of refrigeration system is most common in the food processing industry, in which condenser working conditions greatly influence the overall efficiency of the refrigeration system. Effectiveness of the refrigeration system to a great extent depends on the efficiency and economy of heat rejection by obtaining desirable condensing temperature. Increase in the condensing temperature results in reducing the economy of the refrigeration system. Various influencing factors, including temperature and flow rate of cooling water, heat transfer coefficient and heat transfer surface are very significant. Hence, attempts to optimize the temperature of cooling water along with the cleanliness of heat transfer surface are productive and desirable preventive operations for the economical generation of refrigeration effect towards quality and cost effective supply chain management.

Zum Management der Kondensationstemperatur zur Verbesserung der Effizienz von Kühlsystemen

Die mechanische Rekompression (Entspannung) des Dampfes in Kühlsystemen ist in der Lebensmittelverarbeitungsindustrie weit verbreitet. Die Arbeitsbedingungen des Kondensator beeinflussen wesentlich die Leistungsfähigkeit eines Kühlsystems. Die Effizienz eines Kühlsystems hängt zu einem großen Teil ab von der Effizienz und Ökonomie der Wärmerückgewinnung durch das Erreichen der gewünschten Kondensationstemperatur. Eine Erhöhung der Kondensationstemperatur führt zu einer Reduktion in der Wirtschaftlichkeit des Kühlsystems. Verschiedene Einflussfaktoren einschließlich der Temperatur und der Fließrate des Kühlwassers, des Wärmetransferkoeffizienten und der Wärmeübertragungsoberflächen sind von hoher Bedeutung. Daher sind Ansätze zur Optimierung der Temperatur des Kühlwassers in Verbindung mit der Sauberkeit der Wärmetransferoberfläche produktiv und wünschenswerte vorbeugende Operationen zum ökonomischen Erreichen des Kühleffektes im Sinne eines qualitäts- und kosteneffektiven Managements in der Versorgungskette.

21 Refrigeration (efficiency, condensing temperature) **21 Kühlsystemen** (Effizienz, Kondensationstemperatur)

1. Introduction

In the food processing industry, refrigeration effect is a useful utility for chilling or cooling of products, offices or storage spaces for obtaining and maintaining desired quality and comfort. Among various refrigeration systems, the mechanical vapour recompression type refrigeration system is most popular in industry. This type of system mainly involves throttling, evaporation, compression and condensation unit operations. The refrigerants used in liquid state at high pressure and ambient temperature, when throttled to low pressure, the liquid converts into liquid-vapour phase to lower down the evaporation temperature for extracting heat from the water or air through heat exchanger known as evaporator. The refrigerant leaving the evaporator is in low pressure vapour state containing added heat received in the evaporator. This added heat is required to be rejected in the atmosphere through mechanical compression followed by condensation in heat exchanger popularly known as condenser. How effectively this added heat is rejected in the atmosphere generally depends on many factors including the condensing temperature. With this view this study was undertaken to evaluate the effects of atmospheric condenser cooling water temperature on the efficiency of refrigeration system.