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A comparison of regional and systemic humoral immune responses to a nematode infection

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1. ABSTRACT

The kinetics of humoral immune response against *Trichinella spiralis* (TS) was characterized with immunofluorescence assay. The mesenteric lymph nodes (MLN) and the spleen of infected rats were examined for concurrent expression of multiple antibody (Ab) isotypes from day 1 to day 15 after infection. The tissues were processed and stained with either a pan-B cell marker (OX33) conjugated with rhodamine (XRITC) or combinations of dual monoclonal Ab probes plus A secondary Ab conjugated with XRITC or fluorescein (FITC). As compared to the uninfected controls, the spleen and the MLN showed significant proliferation of dual-Ab expressing B cells (Debc) on days 5 and 7, respectively. During the immune response, only minimal numbers of B cells expressed single Ab isotype while most B cells expressed more than one isotypes of Ab. When combining all the numbers of Debc within each tissue for each respective days, and comparing those numbers with the total numbers of B cells that were $OX33^+$ in the serial sections of the same tissue specimens, the combined Debc in the spleen were > 6 times higher than the OX33 labeled B cells on day 10, and the Debc in MLN were > 3 times higher than the $OX33^+$ B cells on day 10. Our results thus indicate that the Debc most likely expressed more than two Ab isotypes during the peak days of the humoral immune response to the parasite and this phenomenon occurred in both regional and systemic lymphoid tissues.

2. INTRODUCTION

The linings of the mucosal tissues have the largest surface area of any part of the body (1), which provides mechanical and chemical barriers protecting the gastrointestinal tract, the upper and lower respiratory tracts, the urogenital tract and the oral cavity. Among the cells of the mucosal tissues are various immunocompetent cells, including macrophages, dendritic cells, lymphocytes and other effector cells that help to prevent further invasion of pathogens into the body. The cells lining the mucosa are constantly being exposed to pathogens, including viruses, bacteria and multicellular parasites. Thus, it is due to this constant bombardment of pathogens that vertebrates developed a mucosal immune system.

Among the best-studied mucosal lymphoid tissues are the gut-associated lymphoid tissues (GALT) of the gastrointestinal tract. The GALT comprises the Peyer's patch, the mesenteric lymph nodes (MLN), and a large number of lymphocytes throughout the lamina propria and the epithelium of the intestine (1). It is well established that the major GALT contains an elaborate B cell population. IgA-producing B cells, in particular, have been well studied within these tissues (2-4). Although IgA is the major Ab found in mucosal regions, other isotypes of Ab, such as IgM, IgG, and IgE, can also be present in mucosal tissues, depending on the infectious pathogen (5,6).

Trichinella spiralis (TS) is a nematode parasite capable of infecting the epithelial layer of the intestine in all mammals. Once ingested, the TS muscle larvae (ML) are able to enter the intestinal epithelium and become adult worms (7). In the intestine, the adult worms produce newborn larvae, which then migrate through the mucosal tissues, the mesenteric lymph nodes and further drain into the lymphatic system and the general blood circulation. Upon distribution to the muscle tissues, these newborn larvae bury into muscle cells and eventually become infectious ML (8,9). The parasite completes its life cycle once being ingested by a subsequent host.

Due to its unique life cycle, TS is able to elicit a regional and a systemic immune response. It has been shown that active immune responses by both T cells and B cells can lead to the eventual expulsion of the pathogen from the intestine (10-14). Recent studies demonstrate a rapid humoral response to TS by B cells. These cells are able to produce all isotypes of Abs in the small intestine and the Peyer's patches and co-express multiple Ab isotypes in these mucosal tissues (15-17). This study is designed to explore the relationship and characteristics of the regional immunity represented by the MLN and the systemic immunity in the spleen against the TS nematode pathogen.

3. MATERIALS AND METHODS

3.1. Experimental Animals and Infection

Male or female pathogen-free AO rats of six to eight weeks of age were randomized into seven groups of six to conduct this experiment. Rats were purchased from Harlan-Sprague Dawley (Indianapolis, IN) and maintained at the California State University San Bernardino vivarium, where food and water were distributed *ad libitum*. TS muscle larvae from infected rats were isolated by digesting minced rat carcasses with 1% pepsin hydrochloride at 37 °C for 1 hr. ML were obtained through filtration with cheesecloth and a 200-mesh sieve using 0.85% NaCl. Infection was carried out on day 0 by injecting 2,000 ML per rat orally using a blunt-end feeding needle.

3.2. Tissue Processing and Immunofluorescence Staining

Tissues from the mesenteric lymph node and the spleen were obtained from both control (day 0) and infected rats on days 1, 3, 5, 7, 10, and 15. They were processed using a snap-freeze technique (18). Each of the frozen tissues was cut into 6-μm sections using a Lab Tek Precision Microtome Cryostat (Lab Tek Instruments Co., Westmont, IL.). The tissue sections were then placed on slides coated with poly-L-lysine, and allowed to air dry. Slides were then fixed in 2% paraformaldehyde at room temperature for 20 min and finally washed three times in 1X PBS pH 7.2.

The above tissues were stained using a double–labeling immunofluorescence assay as previously described (17). Briefly, each tissue section was stained for 1 hr at room temperature with 20 μl of optimally diluted (15) monoclonal mouse anti-rat IgA, IgM, IgG1, IgG2a, IgG2b, or IgG2c (Accurate Chemicals and Scientific Corp., Westbury, NY; Zymed Labs Inc., San Francisco, CA), respectively. Following incubation with the primary antibody, the slides were washed three times with 1X PBS pH 7.2 for 5 min each wash. The slides were further stained in dark with a 1:1 ratio of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig [$F(ab)_2$'] and goat anti-rat IgE labeled with rhodamine isothiocyanate (XRITC) [$F(ab)_2$']. These Ab were H chain specific and in the $F(ab)_2$' form. Normal rat serum was used in antibody preparation to prevent non-specific binding of Ab to the Fc receptors. Slides were washed again and mounted with fluorosave. Antibody labeled B cells were examined using a Nikon Optiphot biological microscope with an episcopic-fluorescence attachment, EF-D, containing a B2A 495nm filter specific for viewing FITC, and a G2A 546nm filter specific for viewing XRITC. Single- and double-labeled cells of 20 fields per section of the spleen and mesenteric lymph node for each rat were quantified at 400x magnification.

OX33 monoclonal antibody (a gift from Oxford University to Dr. Ching-Hua Wang) binds to CD45, a pan-B cell marker on rat B cells. This antibody was conjugated with XRITC in this laboratory. Serial sections of the above tissues were stained with optimally diluted OX33-XRITC and washed in the same manner and the overall B cell numbers were enumerated using the fluorescent microscope as described previously.

3.3. Statistical Analysis

The day specific means of different dual Ab-expressing B cell (Debc) combinations were compared

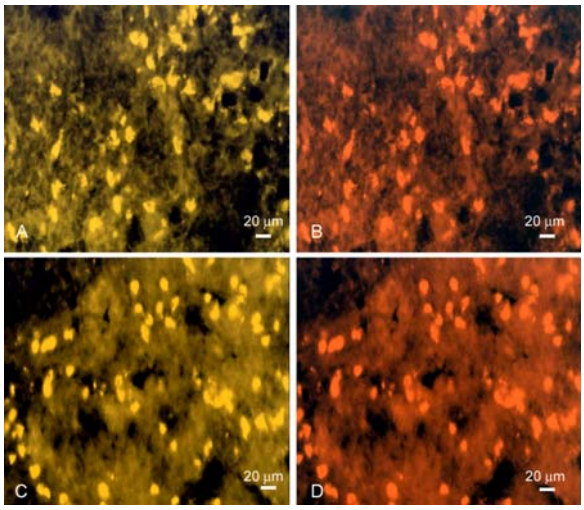

Figure 1. Dual Ab-Expressing B Cells in the Spleen and the MLN of *Trichinella spiralis* Infected Rats. Rats were infected with 2,000 ML on day 0. Tissues were obtained on day 10 and labeled with monoclonal mouse anti-rat IgG1, and FITC-conjugated goat anti-mouse IgG [H&L chain specific $(F(ab)_2$' fragment)]: XRITC-conjugated goat-anti-rat IgE [H chain specific $(F(ab)_2$' fragment)] in a 1:1 ratio. (a, c) IgG1-expressing B cells localized within a field as observed at 400X magnification using a 495nm filter specific for viewing FITC. (b, d) IgE-expressing B cells localized within the identical field as observed at 400X magnification using a 546nm filter specific for viewing XRITC. (a, b) spleen; (c, d) MLN. The diameter of the labeled cells is between 10-12 µm.

using a two-factor ANOVA. The day specific means of Debc and the single Ab-expressing B cells (Sebc) were compared using a two-factor ANOVA. The means of OX33-Ab staining data were compared using a one-factor ANOVA for each tissue tested. The effects resulting in significant F values were then subjected to a Student-Newman Kuels test to determine significant differences between means. q values with probability levels <0.05 were considered significant (19).

4. RESULTS

4.1 Dual Ab-expressing Cells in the Spleen and the MLN of TS infected Rats

To determine the kinetics of expression of Ab by B cells, AO rats were infected with 2,000 TS muscle larvae on day 0 and tissues were taken from the MLN and the spleen on days 0 (control), 1, 3, 5, 7, 10, and 15 after infection. These tissues were cryo-histologically processed and then labeled with monoclonal mouse anti-rat IgA, IgM, IgG1, IgG2a, IgG2b, or IgG2c Ab, respectively, followed by FITC-conjugated goat anti-mouse Ig and XRITC-conjugated goat anti-rat IgE Ab. The Ab-expressing B cells were quantified as either single Ab-expressing B cells (Sebc) or dual Ab-expressing B cells (Debc) by immunofluorescence microscopy. The cells expressing IgE appeared bright-red when examined with a 546-nm filter, and the cells bearing IgA, IgM, IgG1, IgG2a, IgG2b, or IgG2c appeared bright-green when examine with the 495-nm filter. Figure 1 shows the IgG1- and IgE-expressing B cells in the spleen and the MLN on day 10 after infection. The FITC-labeled IgG1-expressing B cells are shown to be localized within the spleen (Figure 1a) and the MLN (Figure 1c). The above B cells in the spleen (Figure 1b) and the MLN (Figure 1d) are also shown to express IgE as bound by the XRITC-conjugated goat anti-rat IgE Ab and are visible by immunofluorescence microscopy.

4.2. Kinetics of Proliferation of Dual Ab-Expressing B Cells in the Spleen

The spleen tissues labeled with specific monoclonal mouse anti-rat IgA, IgM, IgG1, IgG2a, IgG2b, or IgG2c Ab, respectively, and goat anti-rat IgE showed a significant proliferation in the number of double-labeled cells by day 5 after infection (Figure 2). This significant increase in Debc was maintained for the remainder of the experiment as compared to the day 0 controls. There were no significant differences for different dual Ab-combinations between respective days of infection throughout the duration of the experiment. IgA:IgE-Debc showed a significant increase over the control (0.27±0.30) on day 5 after infection (5.77±4.01) with a maximum value on day 10 (10.68±3.17, Figure 2a). IgA-Sebc remained insignificant in the spleen throughout the experiment with the maximum number on day 7 (0.28±0.12). IgM:IgE-Debc showed similar results to IgA:IgE-Debc with day 5 (6.17±2.81) being significantly greater than day 0 (0.53±0.33), and day 10 (10.51±3.41) being the maximum day of proliferation (Figure 2b). IgG1:IgE-Debc showed the highest enhancement on day 10 after infection (13.74±3.38), with a significant increase over the control (0.58±0.21) as of day 5 (6.20±3.45, Figure 2c). IgG2a:IgE-Debc also reached their highest proliferation on day 10 (11.21±2.02) with day 5 (5.58±5.44) being the first day of significant increase over the control (0.36±0.40, Figure 2d). IgG2b:IgE-Debc and IgG2c:IgE-Debc showed similar pattern of B cell proliferation. There was a significant increase in B cells on day 5 (5.38±3.72 for IgG2b:IgE-Debc; 5.63±4.46 for IgG2c:IgE-Debc) over the controls (0.43±0.30 for IgG2b:IgE-Debc; 0.38±0.23 for IgG2c:IgE-Debc), and the peak day of proliferation of these cells was on day 10 (12.17±3.36 for IgG2b:IgE-Debc; 11.93±1.54 for IgG2c:IgE-Debc, Figure 2e, 2f). All of the dual Ab-combinations, except for IgG2a:IgE-Debc, showed a significant decrease on day 15 post infection as compared to the peak of proliferation on day 10. When comparing the respective IgE- as well as IgA-, IgM-, IgG1-, IgG2a-, IgG2b-, and IgG2c-Sebc with the Debc, there were significant increases for the dual-expressing B cells over the respective single-expressing B cells, and such significance remained for the duration of the experiment (Figure 2).

4.3. Kinetics of Proliferation of Dual Ab-Expressing B Cells in the MLN

The MLN tissues labeled with specific monoclonal mouse anti-rat IgA, IgM, IgG1, IgG2a, IgG2b, and IgG2c Ab, respectively, and goat anti-rat IgE showed a significant increase in the number of double-labeled B cells

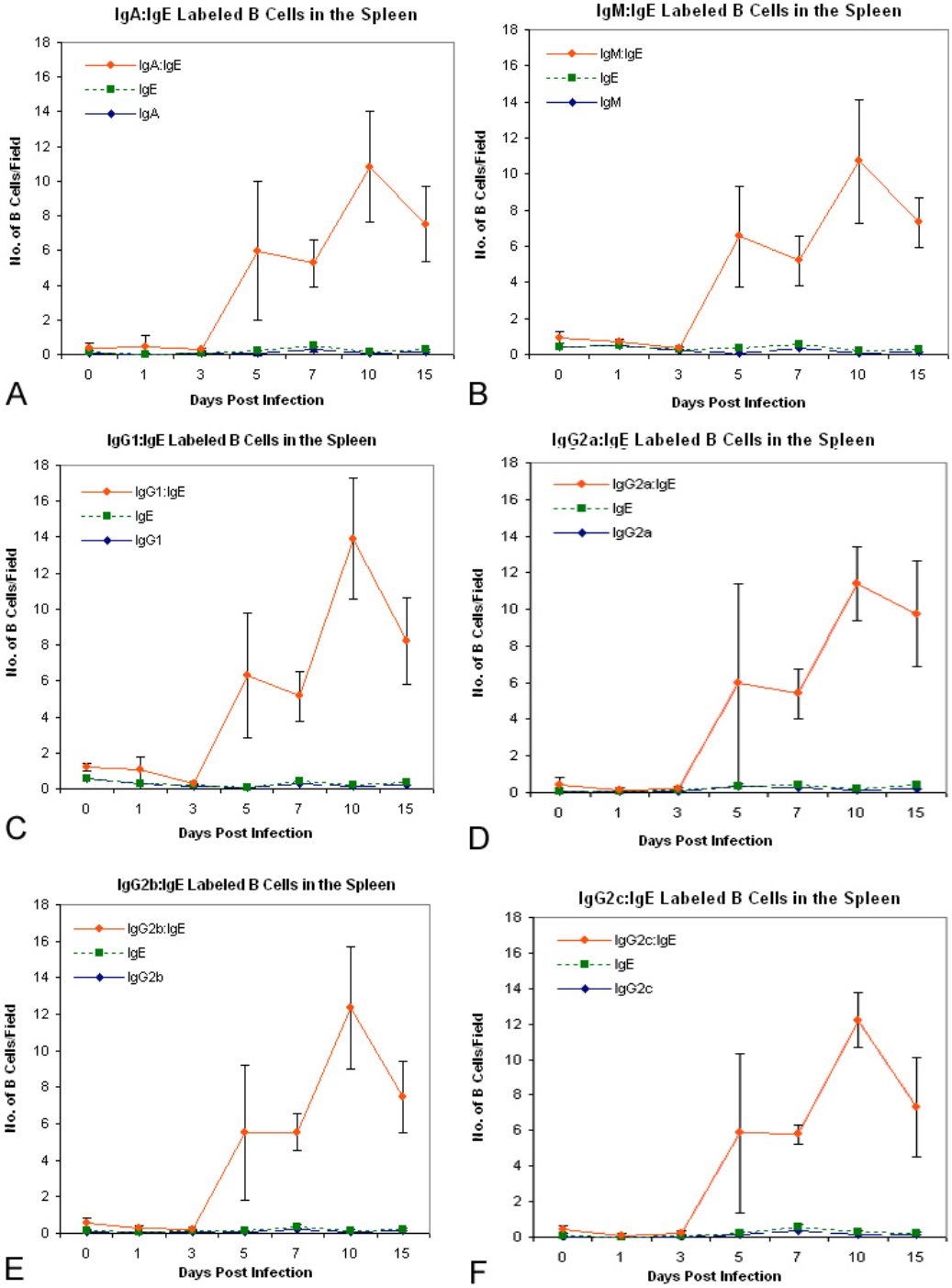

Figure 2. Kinetics of Proliferation of Dual Ab-Expressing B Cells in the Spleen. Rats were infected with 2,000 ML on day 0. The spleen tissues were obtained on days 0 to 15 after infection and labeled with monoclonal mouse anti-rat IgA, IgM, IgG1, IgG2a, IgG2b or IgG2c Ab, respectively, and FITC-conjugated goat anti-mouse IgG [H&L chain specific $(F(ab)_2$' fragment)]: XRITC-conjugated goat anti-rat IgE [H chain specific $(F(ab)_2$' fragment)] in a 1:1 ratio. IgA, IgM, IgG1, IgG2a, IgG2b and IgG2c Ab-expressing B cells in the spleen were quantified at 400X magnification using a 495-nm filter specific for viewing FITC. IgE-expressing B cells localized within the identical fields were quantified using a 546-nm filter specific for viewing XRITC. (a) IgA-expressing B cells; (b) IgM-expressing B cells; (c) IgG1-expressing B cells; (d) IgG2a-expressing B cells; (e) IgG2b-expressing B cells; (f) IgG2c-expressing B cells. (a-f) IgE single-expressing B cells (Sebc) and IgA/E, M/E, G1/E, G2a/E, G2b/E and G2c/E dual Ab-expressing B cells (Debc) in the spleen. Data represent means $\pm$ standard deviation (SD) of 6 rats per field per day.

beginning from day 7 after infection (Figure 3). There were no significant differences for different dual Ab-combinations between respective days throughout the duration of the experiment. IgA:IgE-Debc showed a significant increase over the control (0.95±0.52) on day 7 post infection (4.91±1.50) with a maximum proliferation reached on day 10 (13.23±3.52, Figure 3a). Similar results were found in IgM:IgE-Debc, with day 7 (3.31±2.64) being significantly greater than day 0 (0.39±0.56), and day 10 (13.18±6.34) being the maximum day of augmentation (Figure 3b). IgG1:IgE-Debc began to show a significant proliferation on day 7 of infection (4.39±1.35). Such proliferation continued and peaked on day 15 (9.35±2.40, Figure 3c). Similar dynamics were found among the IgG2a:IgE-Debc and IgG2b:IgE-Debc, with day 7 (3.43±2.28 for IgG2a:IgE-Debc and 3.66±1.53 for IgG2b:IgE-Debc) demonstrating a significant proliferation, and day 10 (8.48±4.01 for IgG2a:IgE-Debc and 10.25±3.58 for IgG2b:IgE-Debc) being the peak day of proliferation (Figure 3d, 3e). The number of IgG2c:IgE-Debc also revealed that day 7 (3.32±1.76) was the first day of a significant increase over the control (1.11±0.39) and day 15 (7.25±2.77) was the peak day of activation (Figure 3f). When compared, there were significant increases for all of the dual-expressing B cells over the respective single-expressing B cells, except IgA:IgE-Debc to IgA-Sebc, as of day 3 of infection, and such significance remained throughout the rest of the experiment.

4.4. Kinetics of Proliferation of $OX33^+$ B Cells in the Spleen and the MLN

To determine the total numbers of B cells in each of the tissues examined, serial sections of the same tissues of the spleen and the MLN were stained with monoclonal mouse anti-rat OX33 Ab conjugated with XRITC. The quantity and the kinetics of B cells were examined by immunofluorescence microscopy and the $OX33^+$ cells appeared bright-red when using a 546-nm filter as shown in Figure 4. The photographs are of samples from the spleen (Figure 4a) and the MLN (Figure 4b) on day 15 after infection. A significant increase in $OX33^+$ B cells was found in the spleen on day 10 after infection (16.85±4.58) over the controls (6.04±1.64), with day 15 (22.58±5.20) being the peak day of proliferation (Figure 5a). The MLN showed a significant rise of B cells over the control (5.35±3.45) by day 5 after infection (13.44±5.62, Figure 5b). B cell proliferation continued in the MLN afterward, with the highest level reached on day 15 after infection (29.41±2.66).

5. DISCUSSION

B lymphocytes proliferating in the small intestine of rats against *Trichinella spiralis* infection have been shown to produce nearly all Ab isotypes at some point during infection. Bell et al. (20) showed that three isotypes of IgG in rats, e.g., IgG1, IgG2a, and IgG2c, were actively produced by B cells against this infection. Other groups have shown drastic increases of IgA (21) or IgE (14, 15) against the same parasite. Based on recent findings (15, 16), it was shown that IgE-producing B cells produced the strongest response against the 9D4 antigen of *Trichinella spiralis*. Therefore, IgE was used as a common Ab marker for testing the dual Ab-expression on B cells in this experiment. Quantitative examination of dual Ab-bearing B cells and single Ab-expressing B cells from the spleen and the mesenteric lymph node of Sprague-Dawley rats are reported. Using a double-labeling immunofluorescence assay, cell surface IgA, IgM, IgG1, IgG2a, IgG2b, and IgG2c were tested, each combined with the common IgE Ab. A separate immunofluorescence assay was also carried out to quantify total B cell numbers within the same tissues to examine the possibility that B cells in rats might express multiple Ab isotypes on the surface during *Trichinella spiralis* infection.

Our previous study demonstrated an early activation and proliferation of dual Ab-labeled B cells in the non-Peyer's patch region of the small intestine (17). Such an increase occurred as of day 3 post infection for all of the Ab isotypes tested. Clearly, our current study indicates that B cell activation and proliferation in the MLN and the spleen lagged behind that of the B cells in the small intestinal tissue. This result confirmed our previous finding in that the intestinal B cells can undergo active proliferation when stimulated to fight off the infection even before the other surrounding lymphoid tissues show any sign of B cell activation. The total B cells detected by the OX33 monoclonal Ab showed a significant augmentation in the small intestine only 3 days after infection with TS (17). Our present study indicates that a significant B cell proliferation occurred on day 5 in the MLN and on day 10 in the spleen after infection with the same dose of ML (Figure 5). This delayed response was expected because the MLN and the spleen would be among the last to receive any signal of infection within the small intestine.

The MLN is a lymphoid tissue that drains the lymph derived from the Peyer's patch and the small intestine. Consequently, activated T cells and B cells from the small intestine, and the potential antigens secreted from the pathogen would have to migrate or divert into the MLN before a local activation and proliferation could ensue. The spleen is a major organ of the systemic lymphatic system, and therefore activated T helper cells and the dual Ab-expressing B cells from the local mucosal region of the small intestine would have to disseminate systemically before activation could occur within this area. Another possible reason for the delayed response within these two lymphoid tissues could be related to the reproduction and systemic migration of TS newborn larvae. As the larvae migrate out of the small intestine into the various tissues of the body, activation of new B cells would occur. Similarly the intestinal parasite could also secrete antigens which could be drained into the systemic circulation. These antigens could in turn stimulate the MLN, the spleen and other lymphoid tissues, that could lead to a subsequent humoral immune response in these tissues.

Most mucosal B cell research revolves around IgA production within the Peyer's patches of the small

intestine (22-24). This experiment has shown that B cells

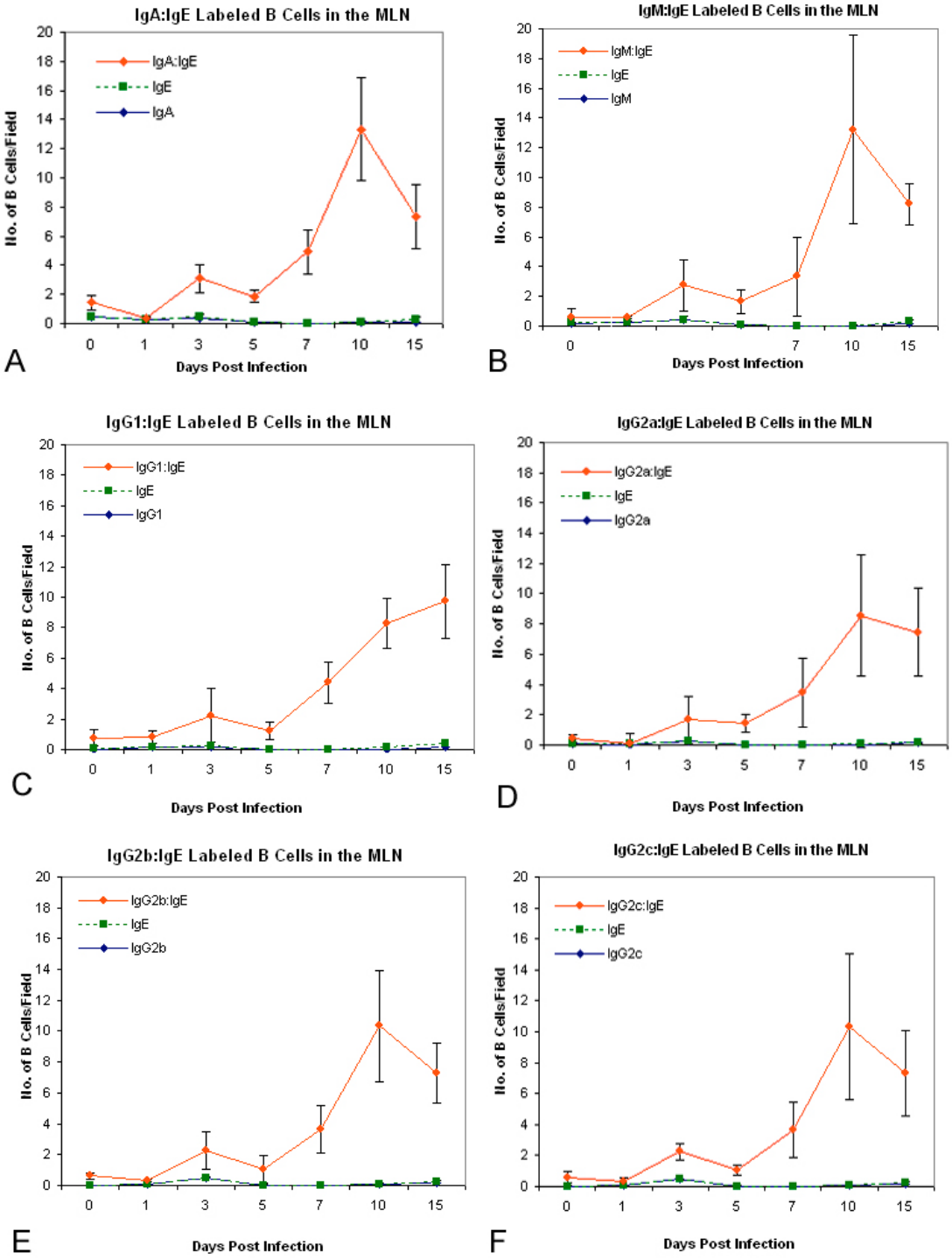

Figure 3. Kinetics of Proliferation of Dual Ab-Expressing B Cells in the MLN. Rats were infected with 2,000 ML on day 0. The MLN tissues were obtained on days 0 to 15 after infection and labeled with monoclonal mouse anti-rat IgA, IgM, IgG1, IgG2a, IgG2b or IgG2c Ab, respectively, and FITC-conjugated goat anti-mouse IgG [H&L chain specific ($F(ab)_2$' fragment)]: XRITC-conjugated goat anti-rat IgE [H chain specific ($F(ab)_2$' fragment)] in a 1:1 ratio. IgA, IgM, IgG1, IgG2a, IgG2b and IgG2c Ab-expressing B cells as well as IgE-expressing B cells localized within the identical fields in the MLN were quantified as described above. (a) IgA-expressing B cells; (b) IgM-expressing B cells; (c) IgG1-expressing B cells; (d) IgG2a-expressing B cells; (e) IgG2b-expressing B cells; (f) IgG2c-expressing B cells. (a-f) IgE Sebc and IgA/E, M/E, G1/E, G2a/E, G2b/E and G2c/E debc in the MLN. Data represent means $\pm$ standard deviation (SD) of 6 rats per field per day.

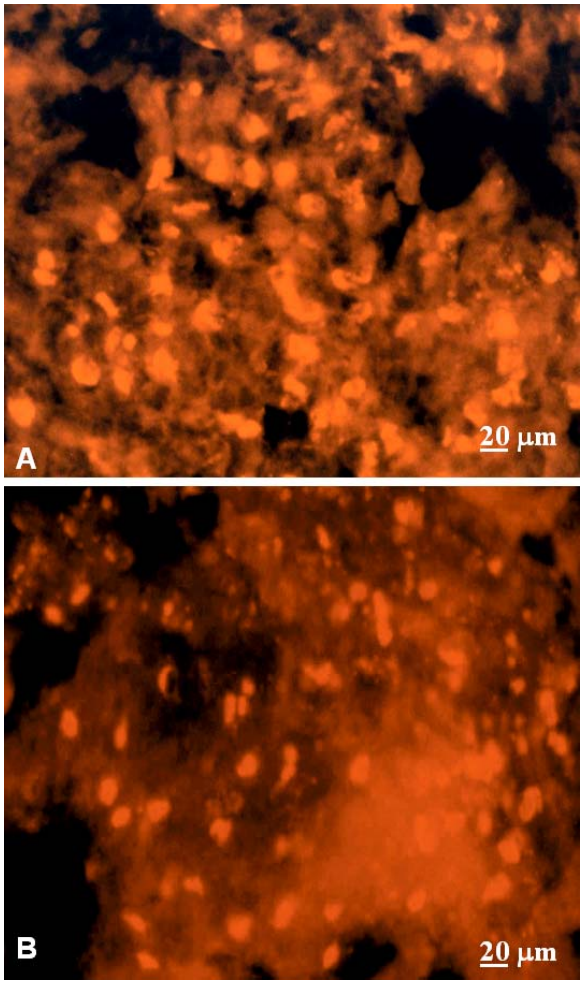

Figure 4. OX 33^{+} B Cells in the Spleen and the MLN. Serial sections of the above spleen and MLN tissues were obtained and stained with monoclonal mouse anti-rat OX33 Ab conjugated with XRITC. The photographs are of samples from the spleen (a) and the MLN (b) on day 15 after infection.

expressed all the different Ab isotypes after infection with *Trichinella spiralis*, confirming the results from our previous study examining the intestinal tissues (17). Our results have also shown that IgA was not the only Ab isotype produced, and that IgA did not even appear to be the major Ab isotype generated against this infection in any of the tissues tested. As would be expected from a parasitic infection and from previous studies (14, 15), IgE would appear to be the major single Ab produced by B cells against TS. In this experiment though, all the other Ab isotypes were also expressed in similar quantities to that of IgE. Therefore no single Ab isotype produced by B cells dominated any other Ab isotype against this nematode infection.

When comparing the kinetics of Debc in the small intestine (17) with that in the spleen and the MLN (Figures 2, 3), we also observed a delayed pattern of proliferation in the Debc in the latter two tissues. The small intestine showed a significant increase in these Debc on day 3 after infection whereas such significant increases occurred on day 5 in the spleen and on day 7 in the MLN. It may be that Debc became activated earlier in the spleen, Or, it could be that the secreted antigens from the intestinal parasites reached the spleen faster than the MLN via the blood circulation. However, when comparing the total OX33-stained cells in these tissues, the spleen first showed a significant increase on day 10 whereas the MLN demonstrated such an increase on day 5. As expected, our results show that total B cells were activated earlier in the MLN than the spleen. Another interesting phenomenon was that the kinetics of Debc in the spleen showed a pattern with twin peaks of proliferation, e.g., on days 5 and 10. However, the MLN only showed a single peak on day 10, with the exception of IgG1/IgE Debc (Figures 2, 3). This twin-peak pattern could be due to stimulation by different antigens from the parasite. The first might be from the intestinal stage of the parasite and the second might be from the migrating newborn larvae that were produced a week after infection. Or, the second peak in the spleen may represent a new round of influx of the Debc from the regional lymphoid tissues into the systemic lymphoid tissue, the spleen, whereas such an influx of Debc from the intestine into the MLN is continuous.

Most studies involving B cells research focus on B cells producing one Ab isotype at a time. There have been only a few studies involving dual-labeled B cells (25, 26), and no study to date on the possible expression of multiple Ab isotypes by B cells to nematode parasites. In this study, activated B cells expressing dual Ab isotypes on the surface were identified and quantified after stimulation by *Trichinella spiralis*. When comparing the dual Ab-labeled B cells to the OX33-labeled B cells in the regional and systemic lymphoid tissues, our data suggest more than two Ab isotypes being expressed on the surface of B cells. This not only confirms what was found in the nonlymphoid region of the small intestine (17), but also strengthens the theory proposed previously that these dual Ab-expressing B cells synthesized more than just two Ab isotypes at any particular point in time. No significant increase in the number of dual Ab-expressing B cells was noticed in the spleen or in the MLN until days 5 or 7 after infection (Figures 2, 3). The number of Debc in the spleen peaked on day 10, which was more than 6 times higher than the total OX33-labeled B cells, and the ratio dropped to less than 3 times on day 15 (Figure 2). In the MLN, the number of Debc was 3 times higher on day 10 whereas it was less than twice the amount of the OX33 labeled B cells on day 15 (Figure 3). This suggests that the B cells in the MLN and the spleen expressed more than two Ab isotypes on the peak days of proliferation and the spleen contained more Debc as compared to the MLN. During most of the infection, however, B cells expressed only up to two isotypes. This was remarkably similar to what was found in the small intestine (17). The strong likelihood of B cells expressing multiple Ab isotypes also suggests active RNA splicing activities in these B cells following antigenic stimulation by TS and activation of T helper cells. Other studies using different cell lines *in vitro* have also

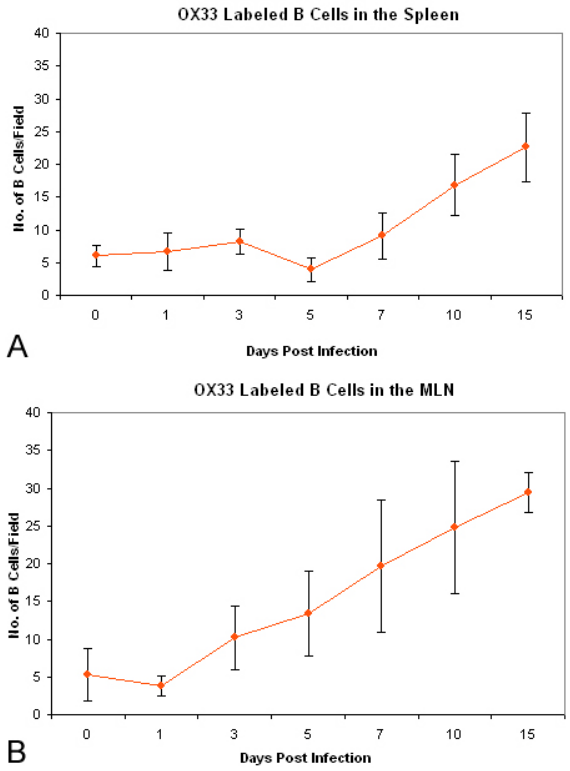

Figure 5. Kinetics of Proliferation of $OX33^+$ B Cells in the Spleen and the MLN. Rats were infected with 2,000 *Trichinella spiralis* ML on day 0. Tissues from the spleen and the MLN were removed on the indicated days of infection as shown in figures 2 and 3 and immunohistochemically stained to reveal $CD45RA^+$ B cells in the spleen (a) and the MLN (b) by staining with the monoclonal antibody OX33. Data represents means $\pm$ standard deviation of 6 rats per field per day.

suggested RNA splicing in mouse (27, 28) and human cells (29, 30), but future research is needed to determine if RNA splicing mechanism also occurs in rats infected with TS. The decline in Debc on day 15 in the small intestine (17) and the spleen and the MLN (Figures 2, 3) is most probably due to the short life-span of these overly committed Debc. Since Sebc did not increase in number towards the final day of testing, it is not likely that these previous Debc became further differentiated into more antigenically committed single Ab-producing B cells. Since we did not use specific antigens in this experiment, we could not determine the antigenic specificities of these proliferating B cells at this point.

One possible explanation for the enhanced Debc could be due to Ab binding to the Fc receptors on various host cells. As neutrophils, eosinophils, basophils, and macrophages were not tested for in this study, Ab secreted by B cells could have been bound to the FcR on these cells. The Ab probes would then bind to the Fc region of the Ab connected to the FcR, thereby giving a positive signal for an Ab-expressing B cell. In rats, most of these FcR-bearing cells have only been shown to express one type of FcR on the surface. Even if they could express more than one FcR, the pairing would not be consistent with the dual-Ab tested for in our experiment. For instance, neutrophils have low affinity FcR for IgG and IgA (31), but have not been found to bind IgE. Eosinophils and basophils express FcR only for IgE (32), and macrophages only subtypes of IgG, but not other types of FcR (33). These cells would then only allow for an increase in the number of single Ab-expressing cells, which remained very low in all of the tissues examined throughout the experiment. The only exception found to date is that of mast cells that have the capability of expressing FcR for IgE and IgG2a in rats (34). In other experimental systems such as mouse and human, FcR specific to more Ab isotypes have been shown (33, 34). Studies with multiple fluorescent markers, or using the fluorescent activated cell sorter analysis to count the various B cells would make the procedures much more accurate and also involve much less time. One could also use more than two fluorescent markers simultaneously to reach more conclusive results as to the theory of multiple Ab production by B cells. Future experiments to determine if RNA splicing is the mode of synthesis of these anti-parasitic Ab could also aid in the better understanding of multiple antibody-expression of B cells.

6. ACKNOWLEDGEMENT

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Key Words: *Trichinella spiralis*, B lymphocytes, B cells, antibody, isotype, antigen, regional immunity, systemic immunity, fluorecein isothiocyanate, rhodamine isothiocyanate, OX33, CD45RA, mesenteric lymph nodes, spleen, FcR.

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